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Studies on the Mitotic Activity of the Corneal Epithelium. Methods. The Effects of Colchicine, Ether, Cocaine and Ephedrin. WILHELM BUSCHKE, JONAS S. FRIEDENWALD AND WALTER FLEISCHMANN 143

Electrocardiographic Changes Associated with Thiamine Deficiency in Pigs. MAXWELL M. WINTROBE, PAUL ALCAYAGA, STEWART HUMPHREYS, AND RICHARD H. FOLLIS, JR. 169

The Plasma Proteins in Disseminated Lupus Erythematosus. ALVIN F. COBURN AND DAN H. MOORE 196

Scapulectomy and Cleidectomy in the Rat. LORING W. PRATT 223

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STUDIES ON THE MITOTIC ACTIVITY OF THE CORNEAL EPITHELIUM

Methods. The Effects of Colchicine, Ether, Cocaine and Ephedrin[1]

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The growth activity of the corneal epithelium is of interest in respect to the healing of corneal wounds and abrasions. Metabolic disturbances of various types such as are produced by deficiencies of vitamin A, riboflavin, tryptophane commonly produce pathological changes in the cornea. Knowledge of the growth activity of the corneal epithelium in these affections might be of considerable interest. With these considerations in mind the present study was undertaken, the immediate purpose being to discover whether reliable indices of the various phases of growth activity in this tissue could be obtained. The present paper is a report of the methods that have been developed so far to facilitate such a study. The use of these methods is illustrated by their application to the study of the effects of several pharmacological agents on the mitotic activity of the corneal epithelium.

EARLIER STUDIES ON THE GROWTH ACTIVITY OF THE CORNEAL EPITHELIUM

Extensive studies have been made on the time required for the covering of artificial epithelial defects of the cornea. The early literature on this phase has been comprehensively reviewed by Jusélius (1). The effects of temperature (1), intraocular pressure (2), inflammation (1, 2), interruption of vascular and nerve supply (2), vitamin A and carotene (3, 4), cocaine (1), and dionin (1, 2) have been studied. Cocaine has been reported not only to inhibit the rate of healing but also the mitotic activity following superficial injury (1).

[1] This work was supported in part by a grant from the John and Mary R. Markle Foundation.

[2] Supported by a grant from the Commonwealth Fund.

According to several authors the covering of a small epithelial defect takes place actually by a migration of cells into the abraded area, while—according to Juselius (1)—a preparatory "karyokinetic wave" begins at the periphery of the cornea and travels from the periphery toward the abraded area. Similarly complicated phenomena have been observed in tissue cultures: According to Jacoby (5) and others, the same agent may stimulate both migratory and mitotic activity in tissue cultures. Other accessory factors (position of the cell in culture mass, cell density, oxygen tension) may determine whether migration or mitosis of a particular cell takes place. The epithelium of the cornea has been shown to retain its ability to cover small defects for as long as 24–48 hours after removal from the body (6).

Studies on corneal mitosis apart from the problem of wound healing have not been extensively pursued in mammals, though mention should be made of investigations in connection with the problem of mitogenetic rays by Gurwitsch and Anikin (7). An interesting and extensive study has however been made by Kornfeld (8) and Politzer (9) on amphibia larvae (salamandra maculosa). The number of mitoses in the corneas of these larvae were counted at various times. In addition "phase curves", i.e., curves representing the per cent frequency of individual phases of the mitotic cycle were constructed. These studies on amphibia larvae were made through the use of flat preparations of the whole corneas. That the methods developed by these authors form a powerful tool is shown by their ability to demonstrate effects on the corneal mitosis rate of hunger and food intake, temperature variations, exposure to X-rays and to various dyes (Neutral Red, Nile Blue, Brilliant Cresyl Blue), to alcohol, ether and other anesthetics. These methods seemed so fruitful that we were encouraged to apply them to the study of mammalian tissue. Most of our experiments were done on rats.

I. MITOSIS COUNTS ON RATS' CORNEAL EPITHELIUM. FLAT PREPARATION METHOD

Procedure. Preliminary experiments indicated that flat preparations of the rat's cornea satisfactory for mitosis counts and for differentiation of the various phases of mitosis could be obtained only if the histological technique was carefully controlled. The method which has given satisfactory results is, therefore, reported

in detail. In these preliminary experiments it was found that the number of mitoses varied greatly from periphery to the center of the cornea but was approximately the same in all meridians. The counts were, therefore, made on a meridional strip prepared as follows:

Flat Preparation Technique on Rats' Cornea. The eye is fixed in a mixture of 2 parts 40 per cent formalin and 1 part 80 per cent alcohol for 3–24 hours. After fixation a meridional band 2 mm. wide is cut out along the greatest meridian of the cornea and separated from adherent conjunctiva, iris and ciliary body. A small scleral flange at each end of the corneal strip facilitates further handling of the specimen since the application of any instrument to the corneal epithelium is to be avoided. The tissue is then rinsed in 80 per cent alcohol and placed for one hour in each of the following: 95 per cent alcohol, absolute alcohol, cedar oil. It may be stored indefinitely in cedar oil. This preliminary dehydration and clearing greatly aids in the clarity of the final stained preparation. After the cedar oil the strips are placed for 15 minutes each in absolute alcohol, 95 per cent, 70 per cent alcohol, distilled water and are stained for 20 minutes in a 1:5 dilution of Harris hematoxylin, rinsed in distilled water, differentiated until deep red in acid alcohol (1 per cent HCl) and left until blue in ammonia water. No counterstain is used. After staining, the tissue is placed for 10 minutes each in 70 per cent alcohol, 95 per cent alcohol, two changes of absolute alcohol, and thin cedar oil (clearing oil). After clearing it is mounted on a slide in thick cedar oil (immersion oil), with the convex (epithelial) side upward. Cedar oil is recommended for clearing instead of xylene which tends to make the tissue stiff and less easily flattened under the cover glass. Instead of the 1:5 dilution of Harris hematoxylin a 1:15 dilution may be used, staining for 1½ hour and omitting the differentiation in acid alcohol. Feulgen stain was also used and yielded very distinct nuclear pictures even in flat preparations; the method, however, is less useful in routine studies.—For *counting* the mitoses an ocular net micrometer was used outlining an area which, with the objectives and oculars used measured $\frac{1}{2}$ x $\frac{1}{15}$ mm. in the specimen. The specimen is moved across the microscope field with a mechanical stop so that a meridional strip $\frac{1}{2}$ mm. in width and the whole corneal diameter in length passes through the counting area. Continuous focussing through different layers of the epithelium is necessary in each field counted. The corneal margin can easily be recognized by the presence of a large circular vessel with adjacent ganglion cells just beyond the corneal margin. When the mitoses are very numerous ordinary histological sections may be prepared cutting the cornea meridional. In such case at least five 6 micron sections were counted.

Number of Mitoses. Based on total cell counts in sample areas of different rat corneas we estimate the total number of epithelial cells in the cornea of a rat weighing 80–150 grams as about 2,000,000. Of these approximately 1,400,000 are in the two basal layers where all the mitoses occur. The average number of mitoses based on sample strip

counts in 18 corneas is 5,600. In other words one in every 250 cells of the two basal layers is, on the average found in mitosis. This figure may be called the mitotic index of the tissue. In the tables given below we have given not the mitotic index but the actual number of mitoses counted in a meridional strip 0.11 × 7.0 mm. in area. The area counted is approximately $\frac{1}{50}$ of the whole area of the cornea. To convert the mitosis count into an estimate of the total number of mitoses in the cornea it must therefore be multiplied by 50. To obtain

TABLE I

Differences in Mitosis Counts on the Two Corneae of Normal Rats in Flat Preparations

PROTOCOL NUMBER	RIGHT EYE	LEFT EYE	DIFFERENCE BETWEEN THE TWO EYES IN PROPORTION TO THE HIGHER VALUES
			per cent
OR	115	120	−4
O4	103	123	−16
O5	122	138	−12
O6	137	124	−9
O7	124	109	−12
O8	159	115	−27
O9	121	129	−6
O10	106	103	−3
O11	66	71	−7
mean			−11
σ			13

the mitotic index the total number of mitoses in the cornea is divided by 1,400,000; i.e., the total number of cells in the two basal layers.

Normal Variations in Mitosis Counts. Mitosis counts in adjacent strips of the same histological preparation differ usually by less than 10 per cent but the variation from animal to animal is considerable. Among about 100 rats so far studied counts ranging from 50 to 250 per strip have been encountered. The difference from eye to eye of the same animal is, however, relatively small, as shown in Table I.

On the basis of these data a decrease of 40 per cent or an increase of 66 per cent in an experimental eye as compared with its fellow control eye may be considered significant.

Anatomical Distribution of Mitoses in Corneal Epithelium. In counting meridional strips the mitoses are often found to be somewhat irregularly scattered across the cornea (Fig. 4, plate I), often occurring in clusters of 3–6 fairly closely placed mitoses separated by moderately large areas devoid of mitoses. The possibility of the presence of some agents temporarily stimulating mitosis in one small region must be considered. In addition it is regularly found that mitoses are more numerous in the peripheral regions of the cornea than in the center.

Phase Distribution. Following the lead given by Politzer (9) and Kornfeld (8) in their study on salamander larvae we have made differential phase counts on the corneal mitoses. Fig. 3 (Plate I) shows the characteristic appearance of the various phases in the mitotic cycle. Fig. 1a shows the average phase distribution in 18 normal rat eyes. In general 100 mitoses were counted in each differential count. Approximately one half of the mitoses are normally found in metaphase, indicating that the duration of the metaphase alone accounts for about one half of the total mitotic cycle (exclusive of the resting phase). It must be pointed out that some of the earliest stages of the prophase probably escape observation with our technique and that the total duration of the prophase probably occupies a larger portion of the whole mitotic cycle than is indicated by our phase counts.

DISCUSSION

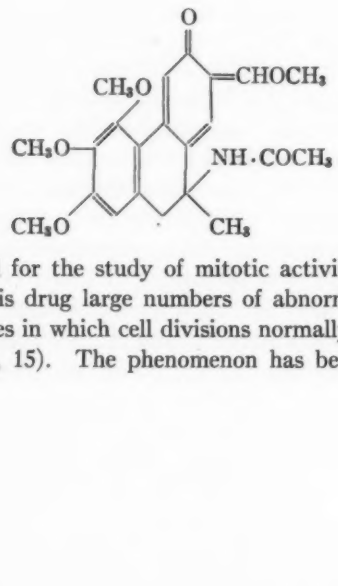
The number of mitoses affords no evidence as to the rate of mitosis except in the extreme limit where, as in the superficial layers of the corneal epithelium the number and therefore also the rate is zero. What is required in addition is some knowledge of the duration of the mitotic cycle. Information on this point can be obtained only with the aid of drugs which interrupt the mitotic cycle at some point, enabling one to observe in successive samples the time required for those phases of the mitotic cycle that lie beyond the point of interruption to disappear from the picture, and for those phases antecedent to the point of interruption to accumulate in number. Absolute reliance cannot be placed upon the results obtained in such pharmacological experiments because one cannot be sure that the drugs applied actually have no effect on the time required by the cell to pass through those

phases of the cycle which appear to be unaffected. Also the time between the application of the drug and the onset of its effect is unknown. However, if concordant results can be obtained by the use of widely differing pharmacological agents one can have greater security in the conclusions to be drawn. In the experiments reported below we have used two drugs, colchicine and ether. The specific effects of each of these will be discussed below in detail, but it may be stated in advance that the estimates of the duration of the mitotic cycle obtained from the application of these two agents are in substantial agreement.

Von Moellendorff (10) who encountered this same problem in his study of mitosis in tissue cultures was able to measure the duration of mitosis by continuous microscopic observation of the growing cells, and, in fact, took continuous motion pictures of the mitotic cycles. Such a technique is inapplicable to the cornea in the present state of our supravital technique, but it is of interest that von Moellendorff's results indicate a duration of the mitotic cycle in the tissue culture which is of the same order of magnitude as that which our pharmacological studies indicate to be the case in the corneal epithelium in vivo.

II. EFFECT OF COLCHICINE

Through the work of Dustin (12) the alkaloid colchicine has been developed



as a valuable tool for the study of mitotic activity. After the administration of this drug large numbers of abnormal mitotic figures appear in all tissues in which cell divisions normally occur in appreciable numbers (11, 15). The phenomenon has been observed in all

vertebrates tested and in many but not all lower organisms. Experiments both in vivo (11, 12) and on tissue cultures (13, 14) have shown that the predominant effect of colchicine is to arrest mitosis in metaphase and not to stimulate resting cells to divide. The most convincing experiments concerning this question are the motion picture photomicrographic studies of Bucher (14) on tissue cultures. He showed that the rate of entrance of cells into mitosis is not increased in colchicine poisoning. With large doses the mitotic rate is in fact somewhat decreased. The arrest occurs apparently early in metaphase and has been attributed to an inhibition of the mechanism of spindle formation (13). The cells in the arrested state show the chromatin accumulated in a dense irregular clump usually surrounded by a halo of clear protoplasm. With larger doses of the drug and prolonged administration many of the cells in the arrested state degenerate and their clump of chromatin becomes fragmented. With smaller doses the effect of the drug has been shown by Bucher to be reversible; after the drug has been removed the arrested cells escape from its influence and complete their mitotic cycle yielding two normal daughter cells. The arrest of mitosis in metaphase under colchicine poisoning has been used extensively in the study of growth activity due to endocrine influences (16, 17, 18) and in studies on tissue regeneration (12) and on malignant growth (11, 12).

Experiments

Histological studies. Doses of 1–3 mgm. per kilo of colchicine were injected subcutaneously or intramuscularly in rats,[3] and the eyes and other tissues were removed at various intervals for histological study. With this dose many animals die in 18–24 hours after the injection. The optimal time for histological study is 6–9 hours after the injection.

Typical "colchicine mitoses" are found in great abundance in the corneal and conjunctival epithelium (Figs. 5, 6, 8, 10, plates I and II). In addition there are a few cells in apparently normal prophase and early metaphase. The cells in "colchicine mitosis" (Figs. 11–14,

[3] The drug obtained from S. B. Penick & Co., Jersey City, N. J., is quite unstable and fresh solutions should be prepared within a few hours of the injection. Even the dry powder deteriorates somewhat in the course of months in spite of storage in the ice box.

plate III) are rather large cells with a brilliantly acidophilic cytoplasm in the periphery, paler clear "mixoplasm" toward the center surrounding a dense mass of clumped globules of chromatin. The globules are 1–2 microns in diameter and the clump looks much like a mulberry (Figs. 11, 14). Except for the clumping of the chromatin, these cells strongly resemble the normal metaphase. In addition, scattered degenerated cells with disintegrated fragments of chromatin are seen (Fig. 12). These cells are often shrunken and irregular in outline. Occasionally they are ruptured and the chromatin fragments and cytoplasm are found discharged. No abnormalities of the resting cells were seen, and no mitoses in other ocular tissues except occasionally in the lens epithelium and in the corneal stroma. The latter may arise from wandering cells which are often present in small numbers in the corneal stroma of rats.

Anatomic Distribution. Confirming the previous studies on normal rat corneae, the mitoses in colchicine poisoning are found almost exclusively in the two basal layers of the corneal epithelium, mostly in fact in the deeper basal layer (Fig. 8). The same is true of the conjunctival epithelium (Fig. 10). The mitoses are most numerous in the peripheral portions of the cornea and are relatively infrequent, but not wholly absent in the central zone.

Number of Mitoses. Since the number of mitoses in the cornea of rats poisoned with colchicine is often very large it is more convenient to estimate the number in sections than in flat preparations. At least 5 sections are counted for each specimen and the average count per section recorded. Table II shows that reliable counts can be obtained in this way when the number of mitoses is large and that the per cent difference between the two eyes of the same animal has the same order of magnitude as with the strip count in the flat preparations of normal eyes. The average count per section is 47. The sections were 6 microns thick. This figure may be compared with the average count of 116 in a strip of cornea 111 microns wide in normal animals, indicating an increase in the number of mitoses of 7–8 fold in approximately 10 hours under colchicine. Allowing for a slight delay in the onset of the colchicine effect and for the presence of a normal number of mitoses in the cornea at the outset of the experiment it follows that under colchicine the number of mitoses accumulated each hour is approxi-

mately equal to the number of mitoses normally present. If colchicine merely stops mitosis in metaphase without affecting the rate at which cells enter mitosis we can estimate the normal duration of mitosis as slightly over one hour.

In Table III are given the mitosis counts in sections of the two eyes of animals, the right eye removed before administration of colchicine, the left eye removed at the specified number of hours after the ad-

TABLE II

Normal Variations of Number of Mitoses in Cornea Sections of Colchicine Poisoned Rats

PROTOCOL NUMBER		SEX OF RAT	WEIGHT OF RAT	DURATION OF COLCHICINE TREATMENT	R. EYE	L. EYE	DIFFERENCE BETWEEN THE TWO EYES IN PROPORTION TO THE HIGHER VALUES
			grams	*hours*			*per cent*
28				12	42	47	−11
32			63	8	36	36	0
38			133	8	24	20	−17
39			165	8	48	61	−21
61	Litter mates	♀	75	10	67	61	−9
62		♂	101	10	36	29	−20
63		♀	95	10	62	70	−11
64		♀	95	10	58	51	−12
65		♀	93	10	46	62	−26
66		♀	88	10	40	38	−5
67		♀	83	10	24	21	−12
68		♀	95	10	34	36	−6
69		♀	84	10	48	55	−13
70		♂	114	10	27	22	−19
Mean							−12
σ							14

ministration of colchicine. The average increase in mitosis count is 300 per cent in $7\frac{1}{2}$ hours exposure to colchicine. In this experiment the control eye was removed under ether anaesthesia immediately before administration of colchicine. We have since found that even a short ether anaesthesia produces a marked inhibition of entrance into mitosis, an effect which persists for about two hours after the end of anaesthesia. There is, therefore, no considerable discrepancy between the rate of mitosis under colchicine as exemplified in the data of Table

III and that calculated in the previous paragraph from the data of Tables II and I.

Phase Distribution Under Colchicine. In a series of animals the eyes were removed at short intervals following the intramuscular injection of colchicine. Flat preparations were made and the number of mitoses counted. Fig. 1b gives the phase distribution computed in per cent of the average normal.

TABLE III

Effect of Colchicine on Number of Mitoses in Rat's Cornea (Sections) 6½-8½ Hours after Parenteral Injection of 2–3 mg./kg. of Colchicine

PROTOCOL NUMBER	WEIGHT OF RATS	NUMBER OF MITOSES IN SECTION OF CONTROL CORNEA	NUMBER OF MITOSES IN SECTION OF CORNEA AFTER COLCHICINE INJECTION	TIME AFTER COLCHICINE INJECTION	ACCUMULATED MITOSES (EXCESS OVER CONTROL EYE)
	grams			*hours*	*per cent*
1		12	46	8	+280
2		7	51	8	+630
34	90	9	28	6½	+210
Co 112		5	10	7	+100
Co 113		6	13	7	+120
Co 114		2	9	7	+350
Co 115		7	9	7	+40
Co 116	approx. 30	5	9	7	+80
Co 117		4	11	7	+175
Co 118		4	11	7	+175
Co 119		4	12	7	+200
Co 120		5	8	7	+60
Co 161	150	4	42	8½	+950
Co 162	140	3	7	8½	+130
Co 163	145	4	28	8½	+600
Co 164	120	5	29	8½	+480
Mean					+286

It is seen that the last three phases of the mitotic cycle disappear almost completely from the picture within 45 minutes of the administration of colchicine. This is in agreement with the conclusion of others that colchicine arrests mitosis in metaphase but allows cells that have already passed through their metaphase to complete the mitotic cycle undisturbed. This gives 45 minutes as an estimate of the duration of the last three phases of the cycle, but this estimate contains

an error, for the time between administration of colchicine intramuscularly and the onset of its effect in the cornea is unknown. We may however take 45 minutes as the upper limit of duration of the last three phases of the cycle, and this is in substantial agreement with the estimate reached in the preceding paragraphs. At any rate it is plain that the effect of colchicine becomes visible in the corneal epithelium within 45 minutes after intramuscular injection.

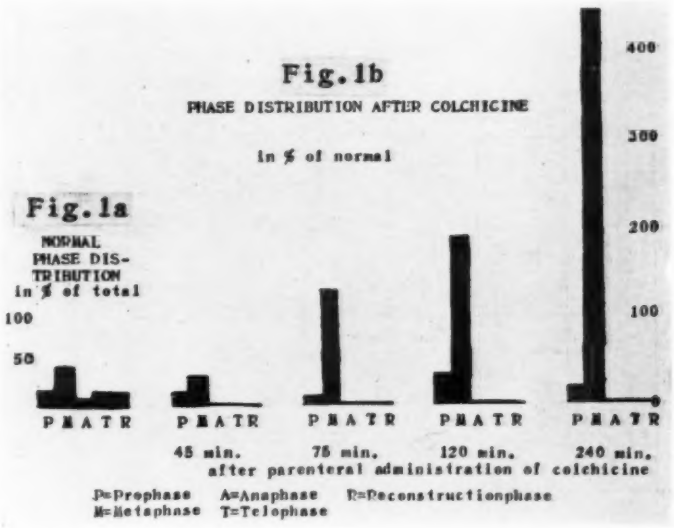


FIG. 1

Local Application of Colchicine to the Eye. In a series of animals colchicine was applied directly to one eye in the form of an ointment, 1 per cent or 5 per cent in vaseline. The ointment was reapplied about every 2 hours. In the opposite eye vaseline without colchicine was similarly applied as a control. As is shown in Table IV there is a considerable accumulation of mitoses in the treated corneas. This is in accord with evidence that colchicine produces its effect by local action as has been shown in other organs and on tissue cultures. The anatomic distribution of the colchicine mitoses was the same after local application as on parenteral administration. Colchicine is

somewhat irritating on local application as evidenced by the presence of some round cell infiltrates in the pericorneal region. It is of interest that the mitosis counts per section in the control eyes are somewhat above normal. The application of vaseline to the eyes of otherwise untreated animals has no effect. It would appear, therefore, that absorption of colchicine by the ocular tissues sufficient to produce a slight effect on the opposite eye takes place.

Effect of Colchicine on the Isolated Surviving Cornea. Since it had been shown that colchicine can produce mitotic accumulation, it was of interest to study the effect of colchicine administered to the isolated excised tissue. If an effect could be obtained it would furnish a measure of the mitotic acitivity in the surviving tissue.

TABLE IV

Effect of Local Application of Colchicine in Vivo on Number of Mitoses in the Corneal Epithelium (Sections)

PROTOCOL NUMBER	SPECIES	NUMBER OF MITOSES IN CONTROL EYE	NUMBER OF MITOSES IN COLCHICINE TREATED EYE	COLCHICINE CONCENTRAT. IN OINTMENT	DURATION OF TREATMENT	EXCESS OF NUMBER OF MITOSES IN COLCHICINE EYE OVER THOSE IN CONTROL EYE
				per cent	*hours*	*per cent*
31	rat	25	50	5	7½	+100
35	rat	16	42	1	5	+162
30	rabbit	6	16	5	7½	+166
37	rabbit	17	44	5	4½	+158

Both beef and rat eyes were used for these experiments. The beef eyes were used as freshly as possible, about 1–2 hours after the animals were killed. The corneas were immediately excised with a narrow scleral rim and separated from other adherent tissue. The rats' eyes were removed immediately after death from animals killed by decapitation without anaesthesia. The excised beef corneas or whole rat eyes were placed in Tyrode solution pH 8.5 with 0.1 per cent glucose, and incubated at 38°C. Small amounts of colchicine were added to some vessels, the others were kept as controls. In the case of the rat eyes one eye of each pair was used as control. No precautions were taken in respect to circulating or ventilating the fluid. It is evident that these conditions are far from physiological and many improvements

TABLE V

Effect of Colchicine on Rat's Cornea in Vitro

AMOUNT OF BATHING FLUID	DURATION OF EXPOSURE	COLCHICINE CONCENTRATION	NUMBER OF MITOSES IN SECTION OF CONTROL CORNEA	NUMBER OF MITOSES IN SECTION OF COLCHICINE TREATED CORNEA
cc.	*hours*			
100	4	10^{-5}	4	15
60	5	10^{-5}	3	13
60	5	10^{-5}	0	5
100	4	10^{-6}	2	9
60	5	10^{-6}	0	4
60	5	10^{-6}	1	3
60	5	10^{-6}	1	5
100	4	10^{-7}	6	4
60	5	10^{-7}	1	1
60	5	10^{-7}	1	0

TABLE VI

Effect of Colchicine on Beef Corneae in Vitro

TIME OF EXPOSURE	CONCENTRATION OF COLCHICINE	STRENGTH OF COLCHICINE EFFECT AS JUDGED FROM CYTOLOGICAL CHANGES AND CRUDE QUANTITATIVE ESTIMATE
A. Variation of concentration		
hours		
7	10^{-5}	++
6	10^{-6}	+
6	10^{-7}	+(+)
6	10^{-8}	±
6	10^{-9}	+
6	10^{-10}	−
B. Variation of time		
1	2.10^{-7}	−
2	2.10^{-7}	+
3	2.10^{-7}	++
4	2.10^{-7}	++
5	2.10^{-7}	++
6	2.10^{-7}	+
$21\frac{1}{2}$	2.10^{-7}	+(+) (Epithelium much damaged)

will probably be required to achieve a mitosis rate in the excised tissues equal to that *in vivo*. Nevertheless the results of these preliminary experiments merit reporting.

An accumulation of mitoses in the colchicine treated rat's cornea was demonstrable with concentrations of the drug down to 10^{-6}, in beef cornea down to 10^{-7}, or even lower (Tables V and VI). The histological appearance of the "colchicine mitoses" was quite characteristic, their anatomical distribution was the same as *in vivo*, but the number of the accumulated mitoses was small compared to that found in the *in vivo* experiments, indicating that the mitosis rate under the conditions of these experiments was only 10–20 per cent of that in the living animal.

DISCUSSION

The analysis of results obtained with colchicine has afforded us one measure of the normal rate of mitosis in the tissue. This estimate is based on the assumption that in the doses given colchicine itself, has no influence on the rate at which cells enter mitosis. This assumption is supported by studies of other investigators on other tissues (12, 13, 14, 15). Nevertheless it would seem wise to support this assumption, if possible, by evidence obtained with some wholly unrelated drug.

III. EFFECT OF ETHER

A number of investigators have found that ether inhibits mitosis. The literature on this subject is well reviewed by Rosenfeld (19). Politzer (9d) in his studies on salamander larvae found not only an inhibition of entrance into mitosis but also a partial arrest in metaphase. Other abnormalities in the mitotic cycle have also been described but with threshold doses the inhibition of entrance into mitosis appears to predominate.

With continuous ether anaesthesia the number of mitoses in the rat's cornea falls practically to zero in from one to two hours and continues at this level for the duration of the anaesthesia. In these experiments the animals were in a closed jar into which ether vapor was introduced. It is not possible, therefore, for us to distinguish between a local effect of the ether on the corneal epithelium and an indirect effect due to the general pharmacological effect of ether. If ether and colchicine are

given simultaneously and the eyes are examined at the end of one hour the number of mitoses is smaller than with colchicine alone, indicating that the ether effect is actually an inhibition of entrance into mitosis. In some experiments with continuous deep ether anaesthesia the onset of the decline in the number of mitoses appeared to be somewhat delayed, suggesting that the progress of cells through the mitotic cycle may be somewhat slowed.

TABLE VII

Effect of Ether Anesthesia (10 minutes) on Mitosis Counts in Rats Corneae (Flat Preparations)

TIME AFTER ONSET OF ETHER ANESTHESIA	MITOSIS COUNT IN		DIFFERENCE BETWEEN THE TWO EYES IN PROPORTION TO EYE BEFORE TREATMENT (CONTROL EYE)
	Control eye (at onset of ether anesthesia)	Second eye of same animal (after ether anesthesia)	
minutes			*per cent*
10	75	73	−3
15	no control eye	74	?
20	66	70	+6
25	53	52	−2
30	67	70	+5
35	32	31	−3
40	78	70	−9
50	127	27	−79
60*	72	16	−78
70	57	0	−100
75	100	18	−82
90	108	43	−60
105	38	144	+278
120	87	123	+41
135	129	101	−22
150	59	83	+59
165	66	93	+41

* Average of two experiments.

The effect of ether in inhibiting entrance into mitosis can be demonstrated even with short duration of the anaesthesia. In these experiments the animals were anaesthetized and one eye was removed immediately. The anaesthesia was continued for 10 minutes and the animals were then allowed to recover. At various times later the animals were killed by decapitation and the second eye was removed. Table VII shows that the number of mitoses counted in the corneal

strips reaches minimal values about 70 minutes after the onset of the anaesthesia, to be followed later by a recovery period in which the normal number of mitoses is considerably exceeded.

It is very instructive to study the phase distribution during this period of decline and recovery. Figure 2 gives the phase distribution calculated in relation to the values in the normal, control eye. It is

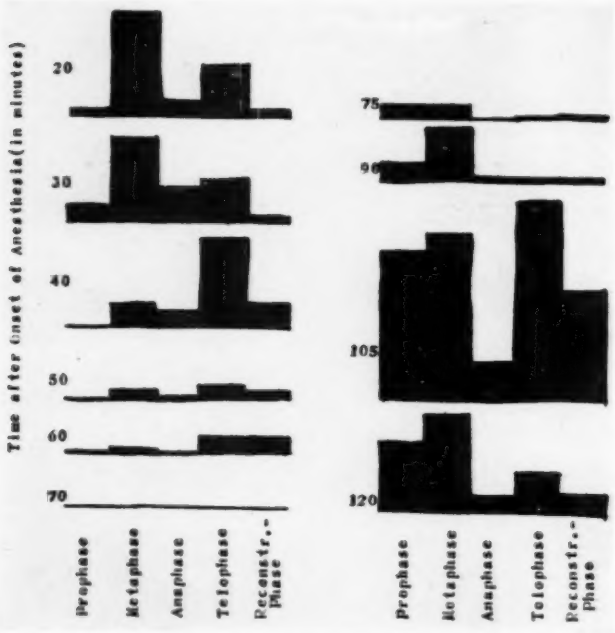


FIG. 2. PHASE DISTRIBUTION AFTER ETHER ANESTHESIA OF 10 MINUTES DURATION (IN RELATION TO VALUES IN CONTROL EYE)

seen that prophases disappear at 40 minutes after the onset of the anaesthesia and telophases at 70 minutes, while recovery begins with the appearance of early phases at 75 minutes and of late phases at 105 minutes. By two hours the number of mitoses and their phase distribution has returned to normal. It is to be seen from these data that the duration of the mitotic cycle during and following ether anaesthesia

is a little over one hour; this is in full agreement with the results previously found with colchicine, and it supports the conclusion that colchicine has little or no effect on the rate of entrance into mitosis.

IV. EFFECT OF COCAINE AND EPHEDRIN

The methods of study reported above provide a means whereby, with a few simple experiments the effects of various drugs on corneal mitosis can easily be unravelled. As examples cocaine and ephedrin were chosen for study. Vaseline ointment containing either 5 per cent

TABLE VIII

Effects of Local Application of Cocaine Hydrochlorid and of Ephedrin Hydrochlorid on the Number of Mitoses in the Living Rat's Cornea (Flat Preparations)

PROTOCOL NUMBER	DRUG	CONTROL EYE (LEFT)	TREATED EYE (RIGHT)	DIFFERENCE BETWEEN THE TWO EYES IN PROPORTION TO LEFT (UNTREATED) EYE
				per cent
Coc 1	Cocaine	65	22	−66
Coc 2	Cocaine	64	4	−94
Coc 11	Cocaine	59	11	−82
Coc 12	Cocaine	59	15	−75
Coc 13	Cocaine	77	17	−78
Eph 1	Ephedrin	63	8	−87
Eph 2	Ephedrin	34	6	−83
Eph 3	Ephedrin	59	4	−93
Eph 11	Ephedrin	38	11	−71
Eph 12	Ephedrin	34	9	−74
Eph 13	Ephedrin	42	8	−81

cocaine hydrochlorid or 5 per cent ephedrin hydrochlorid was applied every 1½ hours to one eye each of a number of 250 gm. rats. The opposite eye was similarly treated with vaseline alone. After 8 hours the animals were sacrificed. Mitosis counts were made on corneal strips. As shown in Table VIII there is a definite reduction in the number of mitoses in the eyes exposed to the drugs. The control eyes in this series show somewhat fewer mitoses than are found in untreated animals. Control experiments using vaseline buffered to the same pH as the drug ointments showed no significant change in mitosis number. The decreased number of mitoses resulting from the applica-

tion of the drugs might be due either to a diminished rate of entrance into mitosis or to an increased velocity of the mitotic cycle. A similar experiment was, therefore, performed on animals with the addition of a subcutaneous injection of colchicine. After 8–12 hours the animals were sacrificed and the corneas sectioned. Tables IX–X show the

TABLE IX

Effect of Local Application of Cocaine on Corneal Epithelium in Colchicine Poisoned Rats (Sections of Cornea)

PROTOCOL NO.	COCAINE CONCENTRATION	DURATION OF TREATMENT	NUMBER OF MITOSES IN EYE TREATED WITH COCAINE	NUMBER OF MITOSES IN CONTROL EYE	DIFFERENCE BETWEEN THE TWO EYES IN PROPORTION TO UNTREATED EYE
	per cent	*hours*			*per cent*
11	1	8	20	37	−46
20	5	8½	7	21	−67
201	5	8½	2	13	−83
202	5	8½	2	5	−60
203	5	8½	1	7	−86

TABLE X

Effect of Local Application of Ephedrin on Corneal Epithelium in Colchicine Poisoned Rats (Sections of Cornea)

PROTOCOL NO.	EPHEDRIN CONCENTRATION	DURATION OF TREATMENT	NUMBER OF MITOSES IN EYE TREATED WITH EPHEDRIN	NUMBER OF MITOSES IN CONTROL EYE
	per cent	*hours*		
204	5	8½	7	6
301	5	8½	5	7
304	5	8½	2	4
451	5	8	1	5
452	5	8	0	4
454	5	8	0	6

mitosis counts on the sections. The number of mitoses in the treated eyes was again less than in the control eyes. Since colchicine arrests mitosis in metaphase it follows that the effect of cocaine and ephedrin cannot be interpreted as due to an acceleration of the mitotic cycle and must be attributed, therefore, to a decrease in the rate of entrance into mitosis.

SUMMARY AND CONCLUSIONS

Methods have been developed for the analysis of mitotic activity in the corneal epithelium of the rat. The number and distribution of mitoses in this tissue have been investigated in the living animal and under supravital conditions, and the rate of entrance into mitosis and the duration of the mitotic cycle have been computed.

The effects of various drugs on the mitotic activity have been studied. Colchicine arrests mitosis in metaphase but has little or no effect on the rate of entrance of cells into mitosis. In the corneal epithelium of the living rat ether, cocaine, and ephedrin all diminish the rate of entrance of cells into mitosis. Under certain circumstances ether appears in addition to increase the duration of the mitotic cycle. The combined use of colchicine together with other drugs affords a simple means of distinguishing between possible effects of these other drugs on the rate of entrance into mitosis and on the duration of mitosis.

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PLATE I

FIG. 3. Photomicrographs of corneal epithelia in various phases of the normal mitotic cycle. Flat preparations of rats' corneas. Harris' Hematoxylin.

FIG. 4. Photomicrograph of flat preparation of normal rat's cornea. To show the somewhat irregular distribution of mitoses in a single field.

FIGS. 5 AND 6. Photomicrographs of rat's cornea after intramuscular injection of colchicine. To show the accumulation of mitoses in metaphase and some scattered nuclear fragments. Flat preparations. Harris' Hematoxylin.

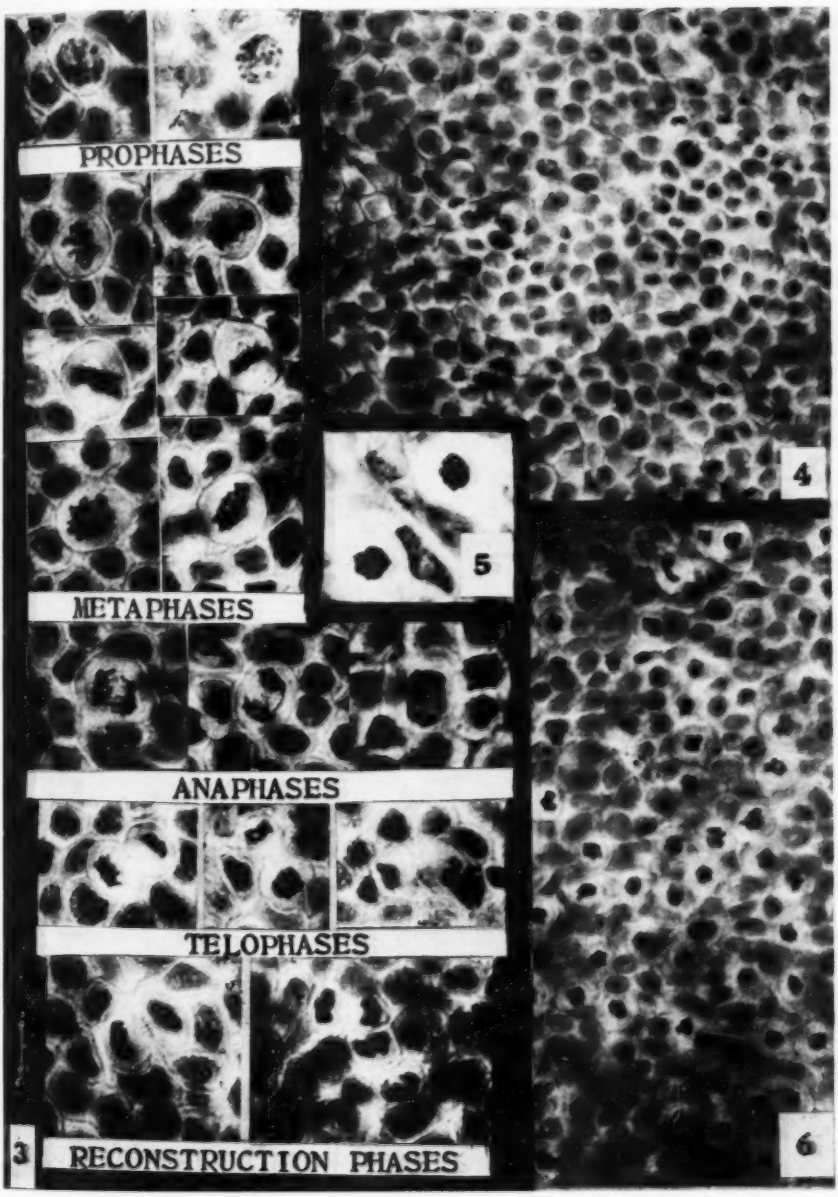
PROPHASES
METAPHASES
ANAPHASES
TELOPHASES
RECONSTRUCTION PHASES
3
4
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PLATE II

FIG. 7. Normal rat's cornea. Section.

FIG. 8. Rat's cornea 8 hours after subcutaneous injection of colchicine. Section. Arrested mitoses in the two basal layers of epithelium.

FIG. 9. Normal rat's conjunctiva. Oblique section.

FIG. 10. Rat's conjunctiva 8 hours after subcutaneous injection of colchicine. Section through 2 adjoining folds.

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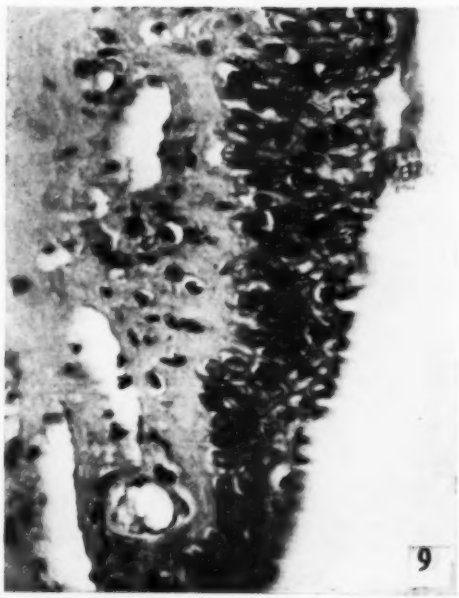
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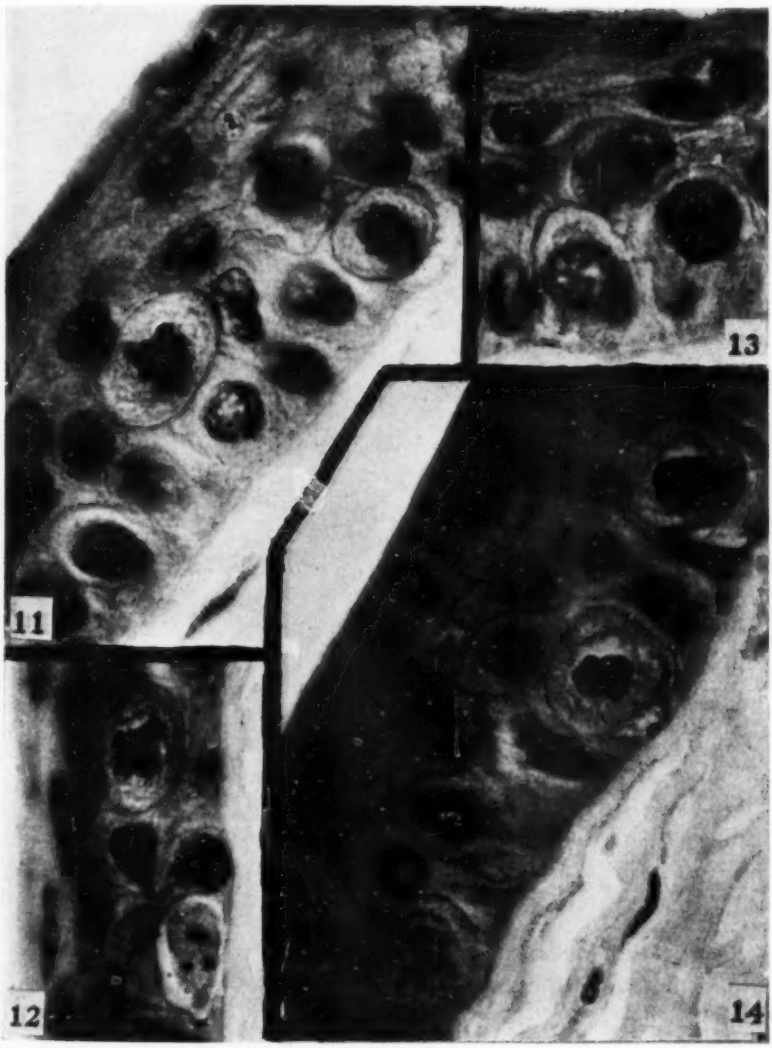
PLATE III

FIGS. 11–14. Photomicrographs of sections through rats' corneal epithelium after subcutaneous injection of colchicine. To show various stages and degrees of the colchicine effect: Arrest in metaphase with thickening and clumping together of the chromosomes (Figs. 13, 14); mulberry-like appearance of the fused masses of chromatin (Figs. 11, 12, 14); disintegration of nucleus with shrinking of cell contents (Fig. 12).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ELECTROCARDIOGRAPHIC CHANGES ASSOCIATED WITH THIAMINE DEFICIENCY IN PIGS[1]

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In earlier reports (1, 2) we have described the effects of thiamine deficiency induced experimentally in pigs and have shown that the chief symptoms of such a deficiency are dyspnea, cyanosis and great weakness, in addition to anorexia, vomiting and moderate impairment of growth. In these animals sudden death occurred and post-mortem examination revealed widespread necroses in the myocardium, pulmonary edema and sometimes pleural effusions, hydropericardium, and hepatic congestion. It is the purpose of this report to describe the electrocardiographic changes which occur in thiamine-deficient pigs.

Cardiac abnormalities have long been recognized as forming an important part of the syndrome of beriberi, a deficiency state associated with the consumption of a diet composed chiefly of carbohydrate. In earlier reports, electrocardiographic changes were rarely mentioned. Aalsmeer and Wenckebach (3) noted only shortening of the PR interval. Keefer (4) observed no characteristic abnormalities except low ventricular complexes. The heart rate varied between 52 and 136 in his cases and the rhythm was normal. However, in cases of pellagra with possibly associated beriberi, Feil (5) noted sinus tachycardia, flattening or negativity of T waves, prolongation of the QT interval, low voltage QRS waves, ventricular or auricular extrasystoles and changes in the electrical axis either to the right or left. Weiss and Wilkins (6, 7) found the most common changes in their cases of nutritional deficiency to be depression and inversion of the T

[1] Aided by grants from the Rockefeller Foundation, the Fluid Research Fund of the Johns Hopkins Medical School, Parke, Davis and Company, the Upjohn Company, and the Fleischmann Laboratories, and carried out in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture.

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waves and prolongation of the electric systole; sinus tachycardia was frequent and at times they observed low voltage of the electrical complexes and premature auricular and ventricular beats. These effects were regarded as due to lack of vitamin B_1. Similar observations have been made by other workers (8, 9, 10, 11).

In experimentally induced thiamine deficiency, Jolliffe and coworkers (12) noted electrocardiographic changes in two out of five human subjects as early as 8 and 11 days, respectively, after they had commenced taking a thiamine-low diet. The changes observed were inversion of T_3 in one of the subjects and, in the other, sinus arrythmia, sinus arrest, changes in deviation of the electrical axis and inversion of T_3. Williams et al. (13, 14) noted diminution in amplitude of all complexes, especially the T waves, the latter even becoming isoelectric or inverted after 12 to 48 days of a thiamine-deficient diet. Bradycardia was found at rest, tachycardia and sinus arrhythmia on exertion, in some instances with premature beats of auricular and nodal origin.

Experimental studies have been reported in pigeons, rats and dogs.[4] Carter and Drury (15) noted bradycardia regularly in pigeons and in some instances found heart block. In the most recent study in this species, that of Swank and Bessey (16), these changes were observed early in the development of deficiency but tachycardia and inversion of the T waves in the second and third leads were found as the deficiency progressed. These workers minimized the significance of bradycardia as a sign of thiamine deficiency since they found that starvation alone would produce this effect.

Drury et al. (17) observed pronounced bradycardia in rats fed autoclaved yeast and failed to obtain this effect in animals fed a complete diet restricted only in quantity. Parade (18), however, and MacDonald and McHenry (19) concluded that bradycardia is due to starvation. Weiss et al. (20), in rats fed autoclaved yeast, observed changes chiefly in the T waves: increase in height, flattening or inversion, high

[4] Electrocardiographic studies in pigs, carried out in the Animal Nutrition Division, Bureau of Animal Industry, U. S. Dept. of Agriculture, are mentioned in the Yearbook of Agriculture, 1942 (Yearbook Separate, No. 1889, p. 820). It is stated that "a lowered heart rate and abnormalities of conduction in the heartbeat" have been observed in thiamine deficiency, but no details are given.

or low take-off and depression of ST segments. Even though there was some slowing of the heart rate when deficiency occurred, the speed of the heart was still so great that it was difficult to recognize changes in PR or QRS intervals. It is of interest that these workers found that atropine or section of the vagus nerves did not abolish the cardiac slowing or the electrocardiographic abnormalities.

Tachycardia of moderate or marked degree developed in eight out of Soldati's eleven dogs fed an autoclaved diet (21), and in a few of the animals the P waves were larger than normal, changes in T waves occurred and there was depression of the ST segment and prolongation of the QR interval. Swank et al. (22) noted electrocardiographic changes in two out of fourteen thiamine-deficient dogs: tachycardia and elevation of the ST segment in all leads in one animal and disappearance of Q_1, prominent Q_3, increasing prominence of T_1, and negativity of T_2 and T_3 in the other.

It is to be noted that a number of the studies just described were made at a time when the multiplicity of the components of the vitamin B group was not fully appreciated. Certainly in the cases of beriberi the nutritional deficiency was not one of thiamine alone. The animal experiments were conducted on the assumption that, by autoclaving yeast, only thiamine is destroyed. Furthermore, the significance of starvation in the production of the observed changes was not given due consideration by certain investigators.

Our own experiments concerning thiamine deficiency in pigs were not commenced until the newer B vitamins had been discovered and it was found possible to raise these animals satisfactorily on a diet in which the B vitamins were supplied in crystalline form rather than in yeast (23). It then became possible to produce thiamine deficiency quite precisely by altering the quantity of thiamine chloride in the crystalline mixture rather than by attempting to destroy it by autoclaving yeast.

As the observations to be described will show, the pig is remarkably sensitive to a lack of thiamine and very pronounced electrocardiographic abnormalities as well as striking histologic changes (2) take place when subminimal amounts of thiamine are supplied. Whereas heart failure has not been found or was overlooked in many experiments concerned with vitamin B_1 deficiency which have been reported

heretofore, with the notable exception of Swank's recent studies (16, 22), heart failure was a prominent symptom and the usual cause of death in our thiamine-deficiency animals.

EXPERIMENTAL PROCEDURE

Full details concerning the experimental methods have been given in earlier reports (1, 24, 25). Three pigs (7-08, 7-09, 7-41) were fed the basal diet (casein 26.1 per cent, sucrose 57.7 per cent, lard 11.0 per cent, salt mixture 5.2 per cent) supplemented with Mead's Blended Oil (vitamins A and D) and a mixture of 8 synthetic B vitamins, all in ample amounts except thiamine. The quantity of thiamine was modified from time to time as will be indicated below. The quantities of the vitamins other than thiamine which were given, were as follows: riboflavin 0.12 mg., niacin 1.20 mg., pyridoxine hydrochloride 0.20 mg., choline chloride 10.0 mg., calcium pantothenate 0.50 mg., inositol 0.10 mg. and para-aminobenzoic acid 0.10 mg. per kg. body weight daily. This dose was given orally by capsule. Each pig was fed one "kilo unit" (36.4 gm.) of the basal diet, furnishing about 152 calories, or a fraction thereof as indicated later, per kg. body weight daily.

In order to compare the effects of thiamine deficiency produced as indicated above, with the changes induced by feeding autoclaved yeast, six pigs were fed autoclaved yeast instead of the crystalline mixture of B vitamins. The yeast used was Mead's brewers' yeast. Some was autoclaved for 6 hours at 20 pounds pressure without any preliminary treatment ("dry autoclaved" yeast). "Alkaline" autoclaved yeast was prepared as follows: 400 grams of yeast were mixed with 600 ml. 0.1 N sodium hydroxide and autoclaved at 20 pounds pressure for 6 hours; 200 ml. 0.3 N hydrochloric acid was then added for neutralization. This was then dried. These two methods of autoclaving were employed because each method has been used by various workers. Three pigs (7-11, 7-14 and 7-40) rereceived the "dry autoclaved" yeast and three (7-12, 7–13 and 7–42) were given the alkaline treated material. All animals were given per kg. body weight per day that amount of autoclaved yeast derived from 3 grams of non-autoclaved yeast. In addition, two pigs (7-13, 7-14) were given crystalline pyridoxine hydrochloride and calcium pantothenate in the amounts stated above.

Electrocardiograms and other studies were made also on

(A) 8 pigs fed the basal diet supplemented with
  (a) 8 synthetic B vitamins including thiamine in adequate amounts (0.25 mg. per kg. body weight daily) (pigs 7-25, 7-27, 7-32, 7-36, 7-64, 7-65); or
  (b) dry, brewers' yeast which had not been autoclaved, 3 grams per kg. body weight daily (pigs 7-15, 7-16).

  Two of the above pigs (7-64 and 7-65) were given limited amounts of food in order to study the effects of inanition.

(B) 14 pigs given adequate amounts of thiamine but lacking various other B vitamins (riboflavin, niacin, pyridoxine, or pantothenic acid).

From time to time the level of the blood pyruvic acid following the administration of glucose, and occasionally thiamine excretion and thiamine tolerance tests (1) were carried out in the pigs. These served as indices of the state of thiamine deficiency.

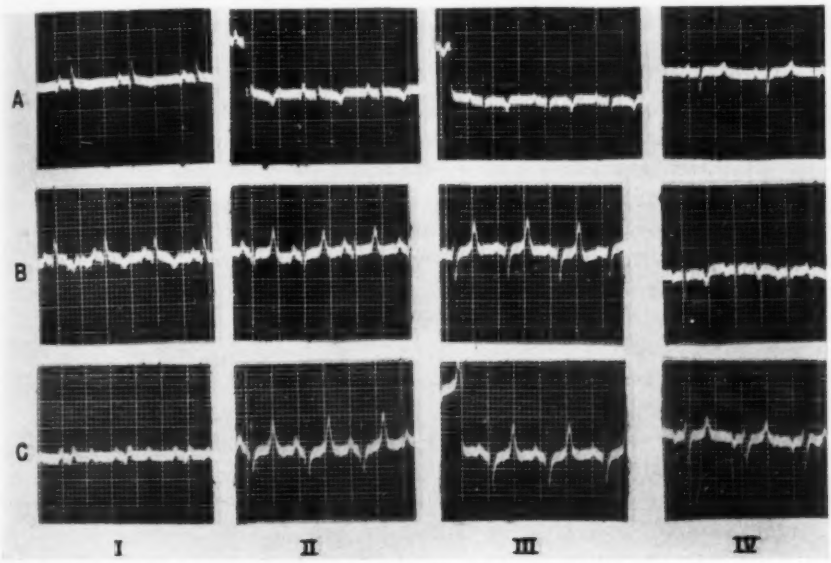

FIG. 1. Electrocardiograms in two normal, well-nourished pigs.

A. Pig 7-27, age 102 days, receiving 8 crystalline B vitamins in adequate amounts. The heart rate is 120, the rhythm sino-auricular. Normogram. The P waves and QRS complexes are normal. T_1, T_2, and T_3 are inverted, T_4 is upright.

B. Pig 7-16, age 105 days, receiving brewers' yeast (not autoclaved). The heart rate is 160, the rhythm sino-auricular. Levogram. Normal P waves and QRS complex. T_1 inverted, T_2 and T_3 upright, T_4 diphasic.

C. Same animal 163 days later. Rate 140, slight sinus arrhythmia. P waves and QRS normal (the low amplitude of QRS in lead I was a common findings in the pigs). All T waves are upright.

For electrocardiography the pigs were placed in a suspension apparatus which allowed them to rest horizontally, supported by canvas straps passing under the abdomen, chest, neck and snout and with their limbs hanging downwards between the straps. The portable Sanborn "Cardiette" with copper electrodes 2 by 6 cm. in size, was used. The electrodes were applied to the posterior (palmar) side of

the legs with the aid of electrolytic paste and rubber bands. For lead IV the electrode was placed approximately 3 cm. to the left of the lower end of the sternum. Electrocardiograms were taken in the afternoon 4½ to 5 hours after the ingestion of food. Five minutes was always allowed for the animal to rest after it had been placed in the suspension apparatus. Electrocardiograms were taken at frequent intervals from the time the pigs were 3½ to 4 months of age.

RESULTS

For comparison with the changes occurring in thiamine-deficient pigs, an analysis and summary are presented in table I of the electrocardiographic findings in (1) healthy, well-nourished pigs, (2) in pigs given ample quantities of all the necessary vitamins but fed quantities of the basal diet insufficient to permit normal growth, and (3) in pigs deficient in one or more of the B vitamins other than thiamine whose growth rate was moderately, or greatly impaired. In figure 1 are shown electrocardiographic tracings of two healthy, well-nourished pigs. In none of the animals listed in table I were shortness of breath, cyanosis or sudden death associated with pulmonary edema observed. Blood pyruvic acid was determined at frequent intervals and was found to be within normal limits. In these animals at autopsy the myocardial lesions we have described in thiamine-deficient pigs (2), pulmonary edema and pericardial, pleural, and peritoneal effusions were not found.

Electrocardiogram in normal pigs

In normal pigs given adequate diets, the heart rate ranged between 130 and 150 beats per minute, averaging 140, being rarely as low as 100 and not often as rapid as 160. The rhythm was sino-auricular with slight or moderate sinus arrhythmia. Generally normograms or a moderate tendency to levograms were observed and only once was a dextrogram recorded. The PR interval ranged between .06 and .12 of a second, the lower value being found usually when the heart rate was rapid, and vice versa. The most constant figure was .09 of a second. The P waves, as measured in lead II, were usually .04 to .06 of a second in duration and 1 to 2.2 millimeters in voltage. The duration of QRS was .04 to .06 of a second, rarely .08 of a second.

The T waves in normal pigs varied so much that it is difficult to present a complete description of them. In general, however, T_1 was

TABLE I

TABLE I

Electrocardiographic Findings in Pigs Given Adequate Amounts of Thiamine

ANIMALS		HEART RATE	RHYTHM	AXIS	PR INTERVAL	P₂		QRS DURATION	T WAVES
Condition	Number					Duration	Voltage		
		per min.			*sec.*	*sec.*	*mm.*	*sec.*	
Normal	6	140 (100–160)	Sino-auricular. Sl. or mod. sinus arrhythmia	Normo- or levogram	.09 (.06–.12)	.04–.06	1–2.2	.04–.06 (.08 rarely)	T_1 −, −, 0 T_2 +, −, ± T_3 +, −, ± T_4 +, +, ±
Inanition, otherwise normal	2	80–140	" "	Normogram	.09–.12	.05–.06	1.5–2.2	.05–.06	T_1 ±, − T_2 ±, +, − T_3, +, − T_4 +
Deficiency other than thiamine. Growth mod. poor	9	100–140	" "	Normo- or levogram Dextrogram 1 animal	.08–.12	.04–.06	1–2.5	.04	T_1 −, −, + T_2 +, −, T_3 +, − T_4 +, +, ±
Deficiency other than thiamine. Growth very poor	5	100–160	" "	Normo- or levogram	.08–.12	.04–.08	1–2.5	.04	T_1 −, −, + T_2 +, − T_3 +, − T_4 +, −, ±

Upright T waves are indicated as +, inverted T waves are represented by −. The types of T waves in the various leads are shown in order of frequency.

more often inverted than upright, whereas in the other leads the T wave was usually upright. Occasionally, inverted T_1 was accompanied

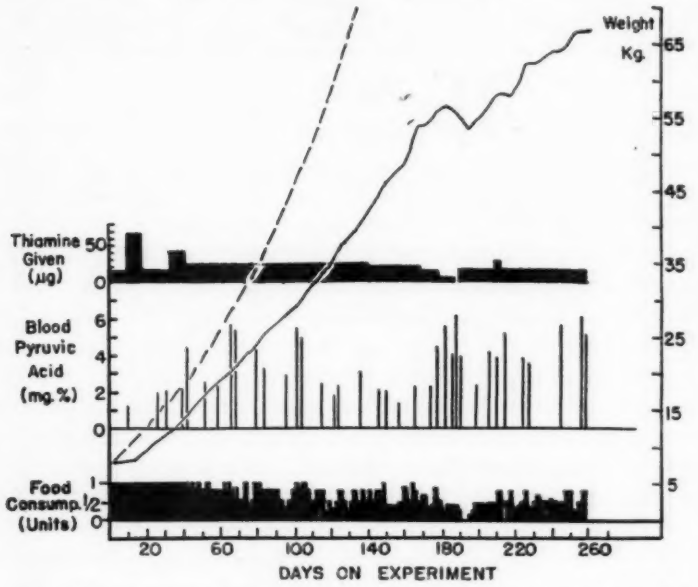

FIG. 2. Food consumption, quantity of thiamine given, rate of growth and blood pyruvic acid in a pig (7-08) given inadequate amounts of thiamine but supplied with adequate amounts of other vitamins. Note the decreased food intake, the impaired growth and the elevated blood pyruvic acid.

The food consumption is indicated in parts of one "kilo unit," which represents 36.4 grams of the basal diet per kg. body weight. With the exceptions mentioned in the text, thiamine was given orally by capsule and is indicated in micrograms per kg. body weight daily. The rate of growth is represented by the continuous line, the interrupted line indicating for comparison the growth of pigs given a complete mixed diet at the Beltsville Research Center. The pyruvic acid content of the blood of pigs not deficient in thiamine has rarely been above 3.0 mg. per 100 ml. and usually ranges between 1.5 and 2.5 mg.

by inverted T_2 and T_3. T_4 was upright with rare exceptions. In table I, as well as in tables II and III, the direction most often found in the various leads is shown first, the next most common type of T wave is given next and the least frequent direction is shown last.

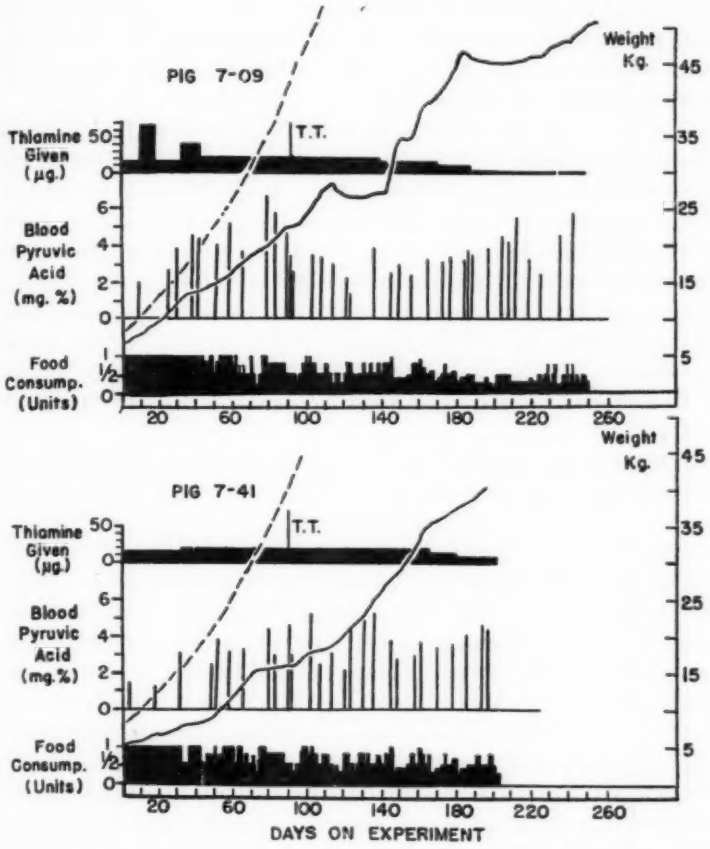

FIG. 3. Food consumption, quantity of thiamine given, rate of growth and blood pyruvic acid in two pigs (7-09 and 7-41) given inadequate amounts of thiamine but supplied with adequate amounts of other vitamins.

Legends same as in figure 2. T. T. refers to an additional amount of thiamine given during a thiamine tolerance test.

Thiamine-deficient Pigs

We have already described the symptoms of thiamine deficiency in the pig (1). Vomiting and anorexia, and sudden death which may

or may not be preceded by dyspnea, inactivity, and cyanosis are the outstanding observable effects. In comparison to that associated with other types of vitamin deficiency, lack of thiamine causes only a moderate impairment of growth (23).

Pigs 7-08, 7-09, and 7-41 were given all the crystalline B vitamins in adequate amounts except thiamine. The experiment was commenced when the pigs were 36 to 38 days of age. By giving small amounts of thiamine chloride, usually 10 to 25 micrograms per kilogram body weight daily (see figures 2 and 3), it was possible to maintain these animals in a state of chronic thiamine deficiency for 258, 254, and 202 days respectively. In figures 2 and 3, the growth curves and food intake of these pigs, and the quantity of pyruvic acid in their blood, are shown. A summary of the electrocardiographic changes is given in table II and some of these changes are illustrated in figures 5, 6, and 7.

In the pigs receiving autoclaved yeast changes similar to those found in the thiamine-deficient pigs supplied only with crystalline vitamins were observed (table III, figures 8 and 9).

Certain findings deserve additional comments.

Heart rate. Bradycardia of pronounced degree occurred in a number of the thiamine-deficient pigs (tables II and III, figure 4). Since it has been claimed that bradycardia results from inanition rather than from the lack of thiamine specifically, it is of interest to compare the heart rate of the thiamine-deficient pigs with those of animals growing poorly as the result of other forms of deficiency, such as pyridoxine, pantothenic acid and riboflavin (table I). As judged by their weights, the general nutritive state of the thiamine-deficient pigs was as good as that of a number of the pigs subjected to other types of deficiency and was actually better than that of some. For example, the weights of pigs 7-08, 7-09 and 7-41 were 42, 31 and 28 per cent of their expected weights at the time of their deaths. The weights of some of the pigs deficient in vitamins other than thiamine which were studied for comparison were as low as 36, 23 and even 13 per cent of the average normal weight for the age of the animals. The heart rate of these animals was never as low as that of a number of those deficient in thiamine. Only in one instance have we observed cardiac rates less than 100 beats per minute in pigs not deficient in thiamine. This was in an animal

TABLE III

Summary of EKG Findings in Three Thiamine-Deficient Pigs

PIG NO.	HEART RATE*	RHYTHM	AXIS	PR INTERVAL	P_2		QRS DURATION	T†
					Duration	Voltage		
	per min.			*sec.*	*sec.*	*mm.*	*sec.*	
7-08	90–120 (140–200)	S-A Sl. or mod. sinus arrhythmia Complete block with ectopic ventricular rhythm	Levogram	.13–.28‡	.06–.11 P_2 notched	1–3.5	.06–.09	T_1 −, ±, + T_2 +, ± T_3 + T_4 +, ±, − (4/15)
7-09	60–120 (150)	S-A Sl. to marked sinus arrhythmia A-V nodal rhythm	Levogram progressively more marked	.10–.28‡	.06–.11 P_1, P_2, notched	1–3.5	.06–.10	T_1 +, ±, − T_2 + T_3 + T_4 − (9) + (8) ± (3)
7-41	80–100 (145–160)	S-A Slight sinus arrthythmia	Normogram	.10–.18‡	.06–.08	1–2.7	.05–.07	T_1 −, ±, + T_2 +, ± T_3 +, ± T_4 + (6) − (5) ± (1)

* The heart rate shown in brackets is that found only when the pig was excited, or following the injection of atropine or when in acute thiamine deficiency.

† The numerals under T_4 indicate the number of times T_4 was found upright (+), inverted (−) or biphasic (±).

‡ First and second degree A-V block.

given adequate quantities of the 8 crystalline B vitamins supplied our control animals, including thiamine, but fed such small amounts of the basal diet that the weight of the animal was kept at 24 per cent of the normal average weight. In this pig the heart rate was 80 to 90 beats per minute and the PR interval 0.10 to 0.12 seconds, which is the upper limit of normal. Later, in this same animal, however, after twelve weeks of a diet furnishing only 20 calories per kilogram and after a reduction in weight to 17 per cent of the normal average weight, the heart rate fell to 24 beats per minute and the PR interval became 0.21 of a second.

It appears, therefore, that both thiamine deficiency and inanition can produce slowing of the heart. Bradycardia, moreover, was not

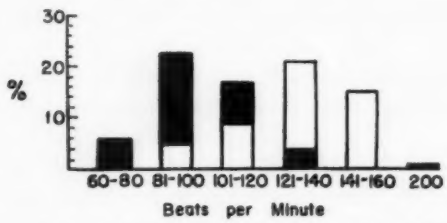

FIG. 4. Frequency of various heart rates in thiamine-deficient pigs (solid columns) compared with those of pigs given adequate amounts of thiamine (open columns).

constant in the thiamine-deficient pigs. Tachycardia occurred when they became excited, after the injection of atropine (see below) and when acute cardiac failure developed in association with thiamine deficiency.

PR interval. Measurements of PR interval were made in leads II or I. Prolongation of the PR interval was observed at all times in pig 7-08 but in the other pigs normal intervals were sometimes noted. In 7-09 and 7-41 there was often only first degree AV block (PR 0.13 to 0.28 sec.). In pig 7-08, particularly, however, the ventricular complex was frequently dropped (second degree auriculo-ventricular block). The combination of bradycardia and dropping of QRS complexes sometimes caused marked prolongation of RR time, even to 2.68 seconds (figure 8, C).

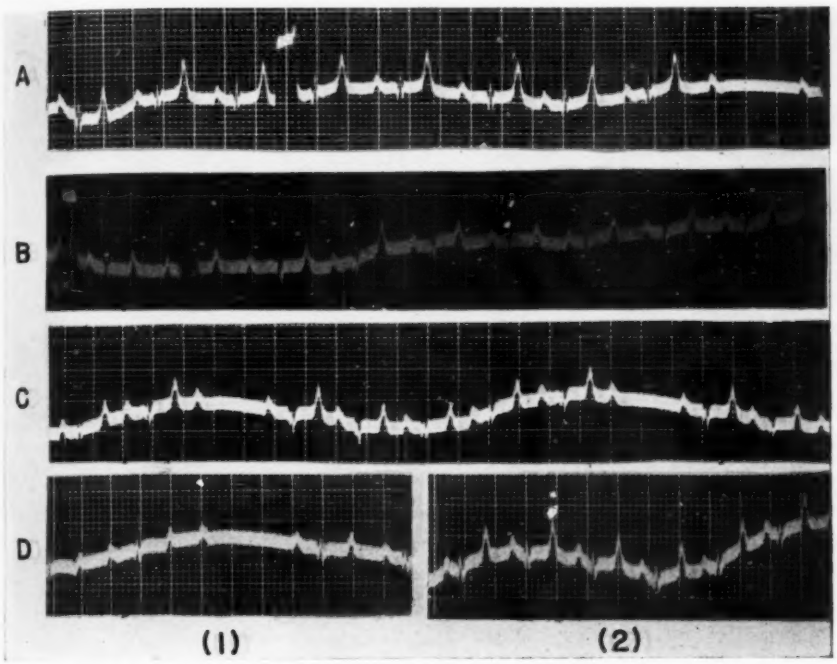

FIG. 5. EKG (lead II) in a thiamine-deficient pig (7-08), receiving 20 micrograms thiamine per kg. body weight daily. Effect of atropine.

A. 89th day of deficiency (age 127 days). Auricular rate 120, ventricular 100–120. PR interval .14–.18 seconds. Second degree AV block. P waves normal. T_1, T_2, and T_3 were upright; T_4 was inverted.

B and C. 116th day of deficiency; B, before eating; C, same day after taking food. The heart rate became somewhat more rapid, the PR interval, which was already prolonged, (.12 to .20 seconds) did not change, but second degree AV block was found in all the leads. The T waves in the other leads were unchanged except that T_1 became variable in direction.

D. 104th day of deficiency. (1) Before and (2) one hour after the injection of 1 mgm. atropine sulphate intramuscularly. The heart rate became more rapid, the PR interval became shorter (.14–.18 decreased to .08–.10) and QRS complexes were no longer dropped. T_1, which had been inverted, became upright.

Other changes in EKG. Other abnormalities in the electrocardiogram of the thiamine-deficient pigs were observed less consistently than the bradycardia, prolonged conduction time and dropping of QRS

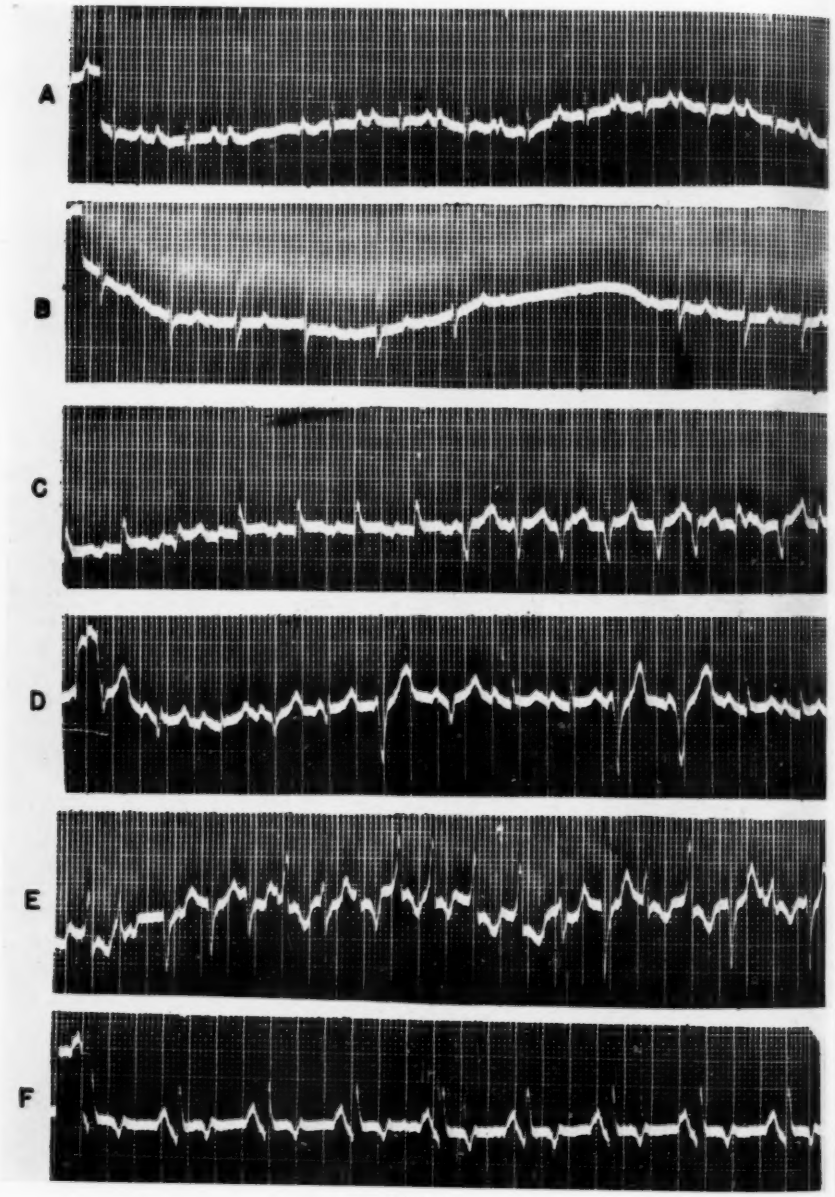

FIG. 6

complexes already noted. In several animals the P waves, especially P_2 became abnormally high and broad (figure 9) and sometimes were notched. Occasionally slight widening of the QRS complex was noted and in a few animals there was a marked shift of the axis to the left. Changes in T waves were very difficult to evaluate owing to the fact that considerable variations apparently occur normally. The most constant abnormality was inversion of T_4. This was probably a significant change because altering the position of the precordial electrode of lead IV only changed its amplitude, and not its direction. In pigs 7-09 and 7-14 exceptionally high T waves were noted. Abnormally elevated ST segments were observed in pig 7-12.

There was slight to well pronounced sinus arrhythmia in most of our animals. Nodal (figure 7, B–E) and ventricular premature beats (figure 6, D), auriculoventricular dissociation with variable ventricular foci (figure 6, E), complete block with ectopic ventricular rhythm (figure 6, C) as well as bigeminal rhythm (figure 7, E) and auricular fibrillation (figure 8, D) were the types of arrhythmia observed in severely deficient animals.

Effect of altering the severity of thiamine deficiency. The customary procedure in our experiments is to give a specified amount of synthetic B vitamins by capsule per kg. body weight and to feed the basal diet separately. The animals thus have no control over their vitamin intake but may consume variable amounts of the basal diet. Since the

FIG. 6. Development of second degree auriculo-ventricular block and then complete block with idioventricular rhythm as thiamine deficiency became more severe in pig 7-08. As the deficiency continued but became less severe, abnormal P waves developed.

A. 188th day of deficiency. Lead II. Prolongation of PR interval (.20–.22 sec.) accompanied at times by dropping of QRS (2nd degree AV block).

B. Same. Lead IV. Two consecutive P waves are not followed by ventricular response. This 3:1 type of second degree AV block occurred after every eleventh ventricular complex. RR time during the block was 1.65 sec. or longer.

C. 190th day of deficiency. Lead I, showing change from first degree AV block to complete block with ectopic ventricular rhythm.

D. Same. Lead II, first degree AV block with frequent ventricular extrasystoles arising from multiple foci.

E. Same. Lead IV. Auricular rate 170, ventricular 200. Complete AV dissociation with variable ventricular foci.

F. 256th day of deficiency. Lead I. Heart rate 100–90. PR interval, .14 sec. P waves abnormal in height (3–3.5 mm.) and duration (.11 sec.) and both upward and downward slopes are notched. The pig died 2 days later.

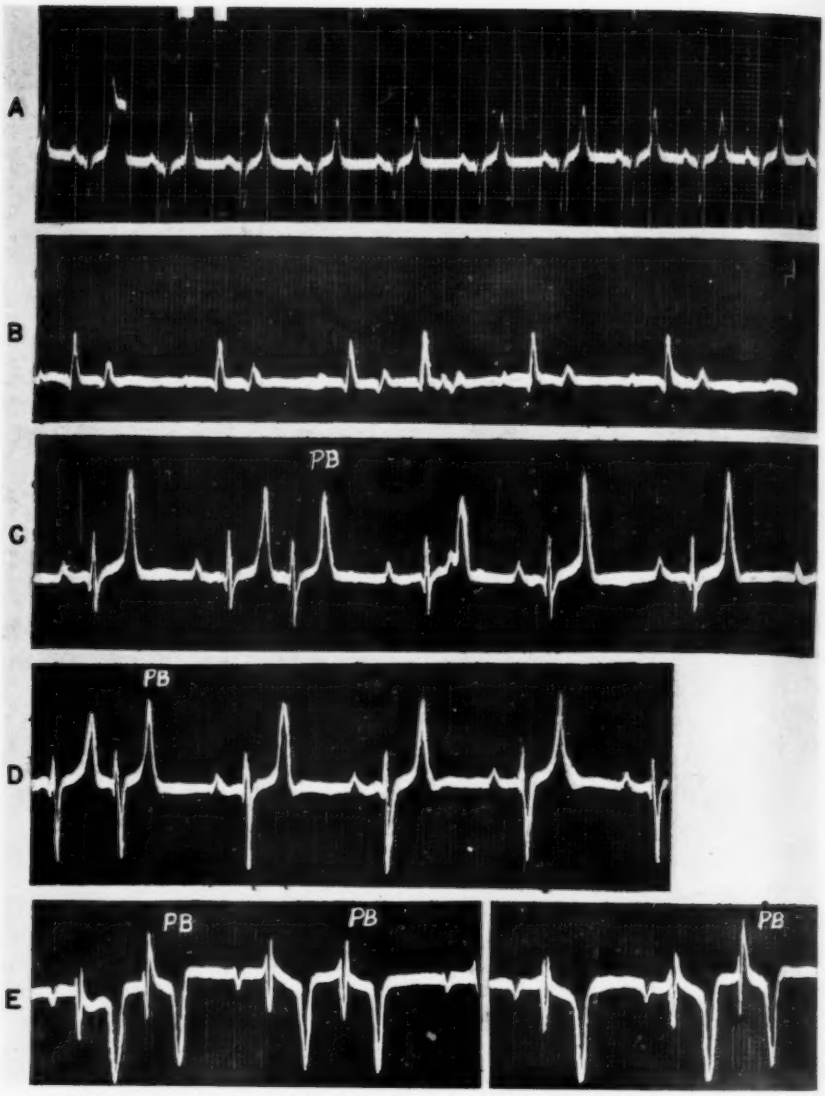

FIG. 7. Development of bradycardia and marked arrhythmia in pig 7-09 as thiamine deficiency progressed.

A. 94th day of deficiency. Lead II. Rate 110. Moderate sinus arrhythmia. PR interval .10-.12 sec. Slight shift of axis to left. T_1, T_2, T_3 were upright, T_4 diphasic.

B, C, D, E. 236th day of deficiency, leads I, II, III, IV, respectively. Rate 70–80. Marked arrhythmia due chiefly to nodal premature beats. Axis markedly shifted to left. PR interval .23–.28 sec. High voltage of QRS. T_1 upright but slightly diphasic. T_2 and T_3 upright and abnormally high (18 and 16 mm. respectively). T_4 inverted. In lead IV (E) there is a tendency toward bigeminal rhythm.

TABLE III
Summary of EKG Findings in Six Pigs Fed Autoclaved Yeast

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Summary of EKG Findings in Six Pigs Fed Autoclaved Yeast

PIG NO.	HEART RATE	RHYTHM	AXIS	PR INTERVAL	P_2		QRS DURATION	T WAVES	REMARKS
					Duration	Voltage			
				sec.	*sec.*	*mm.*	*sec.*		
7-11	60–140 (180 ½ hr. before death)	S-A Slight to marked sinus arrhythmia	Levogram	.08–.26	.04–.06	2	.05–.08	T_1 −, +, ± T_2 +, −, ± T_3 +, −, ± T_4 +, −, ±	Bradycardia—1st and 2nd degree AV Block Auricular fibrillation before death
7-12	120–150	S-A	Normogram to slight levogram	.08–.20	.04–.08	2	.04–.05	T_1 −, ±, + T_2 + T_3 + T_4 +	Slight bradycardia—normal rate 1st degree AV block ST_1 and ST_2 segment elevated R_4 absent Severe myocardial damage
7-13	120–160 (200–220)	S-A Slight sinus arrhythmia	Normogram	.06–.12	.04–.07	2	.04–.06	T_1 +, ± T_2 +, ± T_3 + T_4 +, ±	Slight bradycardia and marked tachycardia Chronic pericarditis at autopsy
7-14	140–160	S-A	Normogram to slight levogram	.12–.17	.06–.10	4	.05–.09	T_1 + ±, − T_2 + T_3 + T_4 +	1st degree AV block Abnormal duration and voltage of P_2 P_2 and P_3 notched
7-40	100–140	S-A Slight to marked sinus arrhythmia	Normogram	.08–.13	.04–.08	1.6	.04–.06	T_1 − +, ± T_2 + T_3 + T_4 −, +	Moderate bradycardia Slight 1st degree AV block Occasionally ST_2 upright T_4 nearly always inverted
7-42	160	Slight sinus arrhythmia	Levogram	.14	.04	.8	.04	T_1 − T_2 + T_3 +	Upper limit of normal rate Slight, 1st degree AV block Pig was excited. Died early in the experiment. This is the only EKG, taken 2 days before death

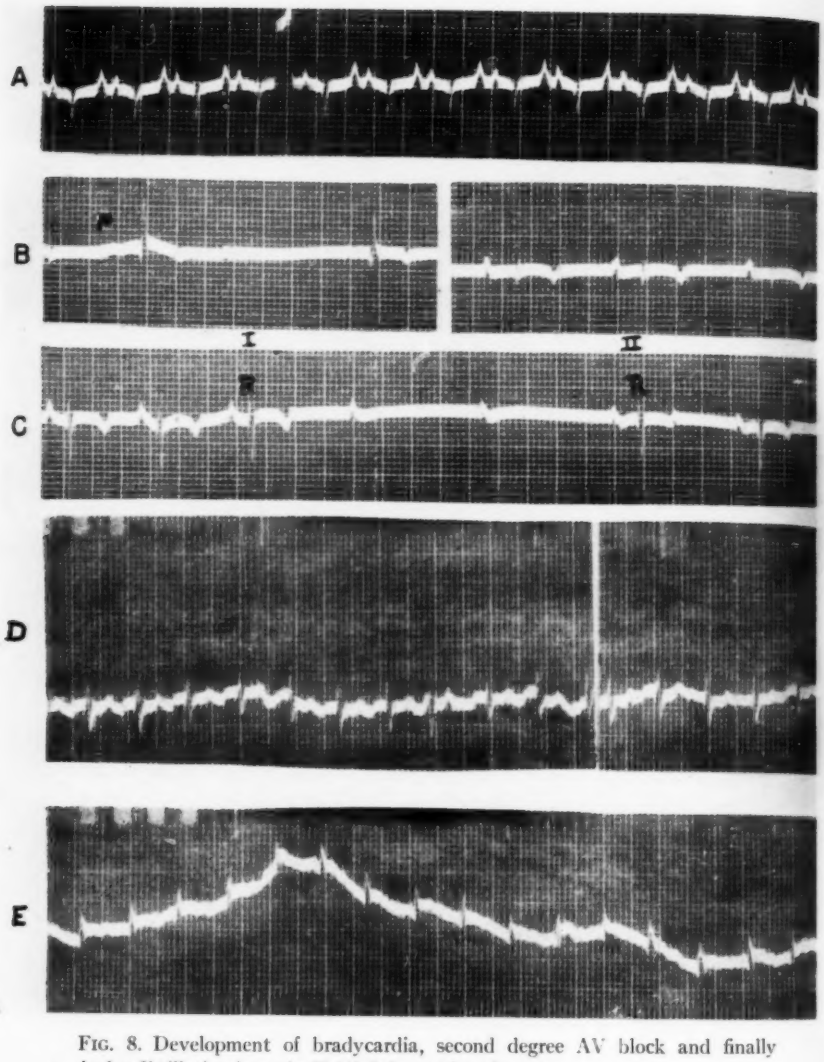

FIG. 8. Development of bradycardia, second degree AV block and finally auricular fibrillation in a pig (7-11) fed autoclaved yeast.

A. 84th day of experiment. Lead II. Heart rate, 140. SA rhythm. PR interval .12–.14 seconds. T_1 was inverted, T_2, T_3 were upright, T_4 was inverted.

B. 222nd day of experiment. Lead I, auricular rate, 90–80, ventricular, 80–60 beats per minute. There was marked sinus arrhythmia. PR interval .12–.26 seconds. Second degree AV block. T_1, T_2 inverted. T_3 was inverted or diphasic. T_4 was upright.

C. Same, lead III. Two QRS complexes have been dropped. RR time, 2.68 seconds.

D and E. 231st day of experiment, leads III and IV respectively.

D. There is tachycardia (rate 180), the auricular waves are totally irregular, and the QRS complexes vary in character (auricular fibrillation).

E. Reversal to normal rhythm. The PR interval is slightly prolonged (0.16 seconds).

thiamine requirement is closely correlated with the caloric intake, the development of anorexia as the result of thiamine deficiency tends to reduce the degree of deficiency. In order to bring about a still greater degree of deficiency than we had heretofore produced, commencing with the 168th day of the experiment pig 7-08 was furnished its thiamine with its food in amounts of 10 micrograms per kilo unit of basal diet. After 20 days the deficiency had become very severe, the animal was short of breath and staggered on moving about. The

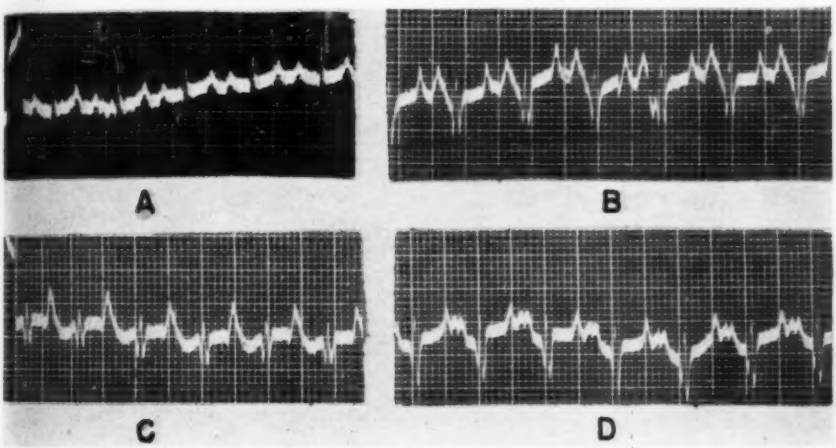

FIG. 9. Abnormal P waves in a pig (7-14) fed autoclaved yeast.
A. 92nd day of experiment.
B. 205th day of experiment. The P waves are abnormally high and wide.
C. 209th day of experiment. The P waves are superimposed on the T waves.
D. 211th day of experiment. The P waves are notched. Q and S are deeper than in B. ST segments normal in all leads.

blood pyruvic acid was 6.19 mg. per cent, a very high value. The most striking change in the electrocardiogram (figure 6, A and B) was the development of a more pronounced second degree auriculoventricular block. The heart rate was still relatively slow (auricular rate 110-120, ventricular 100-110).

At this stage the anorexia became so pronounced that the pig consumed no food whatever. Two days later, no thiamine and no food having been taken in the meantime, tachycardia was found to have

developed (figure 6, C) and complete block with ectopic ventricular rhythm was observed. At this point administration of thiamine by capsule (10 micrograms per kg. body weight) was resumed. Consumption of food commenced 2 days later and 15 days later the heart rate had decreased to 100 and there was only first degree AV block, together with abnormally high and broad P waves. It is of interest that this last abnormality increased progressively until the time of death (figure 6, F).

The production of severe deficiency in pig 7-09 in the same manner as in pig 7-08 was associated with the development of bradycardia (figure 7) and marked arrhythmia.

Effect of atropine. The injection of atropine sulphate (1 mg. intramuscularly) in pig 7-08 was followed by an increase in heart rate and shortening of the PR interval. Before the injection of atropine ventricular complexes had been dropped from time to time. Following injection QRS complexes were no longer dropped (figure 5, D). In pig 7-09 the injection of atropine was likewise followed after 1 hour by a slight increase in heart rate (from 70–80 to 100 beats per min.) and decrease in the frequency of premature ventricular beats. The PR interval became shorter (.16), T_4 which had been inverted became upright.

Similarly, the administration of atropine to a starved animal given ample amounts of thiamine, was associated with a pronounced increase in heart rate (from 24 to 100 beats per minute) and a shortening of the PR interval (from 0.21 to 0.14 of a second).

Pathological findings. All of the pigs receiving crystalline vitamins and three of those fed autoclaved yeast died, apparently of heart failure. The remaining three pigs fed autoclaved yeast were killed. The pathological findings (table IV) were similar to those we have described elsewhere (2). In pigs 7-08 and 7-09 the hearts were particularly large and appeared both dilated and hypertrophied. These organs represented 1.00 and 0.76 per cent respectively, of the total body weight which is well above the normal. In pigs of corresponding age and rate of growth the heart: body weight ratios were 0.39 and 0.44 per cent, respectively. Cellular infiltration and scarring, as well as fresh necroses were found in the auricles and ventricles, including the cardiac septa of all the animals. The cardiac dilatation and hyper-

TABLE IV

Summary of Nutritional and Necropsy Data on Thiamine-Deficient Pigs

PIG NO.	DIETARY SUPPLEMENTS	AGE AT DEATH	DURATION OF DEFICIENCY	FINAL WEIGHT	HEART		PULMONARY EDEMA	SEROUS EFFUSIONS	LIVER
					WEIGHT per cent of body weight	Histologic changes			
		days	*days*	*kg.*					
7-08	Crystalline vitamins	296	258	65.5	1.00	Marked	Present	Pleural	Normal
7-09	Crystalline vitamins	290	254	50.1	0.76	Marked	None	Peritoneal	Ch. passive congestion
7-41	Crystalline vitamins	238	202	40.4	0.64	Very marked	Present	Pleural Pericardial	Normal
7-11	Autoclaved yeast (dry)	273	231	52.4	0.45	Very marked	Present	Pleural	Normal
7-12	Autoclaved yeast (alk.)	147	103	29.6	0.44	Moderate	Present	None	Engorged, no central atrophy
7-13	Autoclaved yeast (alk.) plus B_6, P†	241	204	65.9	0.91	Moderate	None*	Pericarditis	Few focal necroses in center of liver lobules
7-14	Autoclaved yeast (dry) plus B_6, P†	280	226	83.0	0.55	Moderate	None*	None	Normal
7-40	Autoclaved yeast (dry)	244	206	84.0	0.61	Marked	None*	None	Normal
7-42	Autoclaved yeast (alk.)	101	67	15.5	0.58	Slight	Present	Pericardial	Normal

* These animals were sacrificed. The rest died of cardiac failure.

† B_6 refers to pyridoxine, P to calcium pantothenate.

trophy was less marked in pig 7-41 than in pigs 7-08 and 7-09, but the microscopic lesions were even more extensive. Pulmonary edema and serous effusions (pleural 50 to 500 cc., pericardial 20 to 500 cc., and peritoneal 2000 cc.) completed the evidence of cardiac failure.

The histologic changes in the hearts of the three pigs which were sacrificed (7-13, 7-14 and 7-40) were as extensive as in the pigs fed autoclaved yeast which died, but pulmonary edema and serous effusions were lacking. Of all the pigs which were given autoclaved yeast, the most pronounced histologic changes were found in pig 7-11. Except in pig 7-14, in which chronic pericarditis with adhesions were found, the heart: body weight ratio was not as high in the pigs fed autoclaved yeast as in those given only crystalline vitamins as a source of B vitamins other than thiamine.

DISCUSSION

The electrocardiographic changes which accompany thiamine deficiency in the pig appear to be more pronounced than has been described in other animals or in man. They give further evidence of the fundamental importance of this vitamin for the heart. The changes noted, as well as the pronounced anatomical alterations which occur (2), are attributable specifically to a lack of thiamine. Similar electrocardiographic and histologic studies of pigs deprived of pyridoxine, pantothenic acid, riboflavin or nicotinic acid but supplied with adequate amounts of thiamine have failed to reveal abnormalities in the myocardium or in the electrical conduction system of the heart. It is noteworthy that Swank and Bessey (16) have shown that electrocardiographic abnormalities and evidences of cardiac failure in thiamine-deficient pigeons are accompanied by a marked decrease in the cocarboxylase content of the heart muscle.

Although bradycardia has long been regarded as a sign of vitamin B_1 deficiency, Swank and Bessey (16) recently have emphasized tachycardia in this connection and have given starvation credit for the production of bradycardia. The observations of Weiss et al. (20), however, indicate the importance of thiamine specifically. These authors found that the heart rate of thiamine-deficient rats with bradycardia rapidly increased to normal when thiamine was given, even though the animals fasted both before and during the curative response.

Our observations suggest that inanition alone may produce bradycardia. Thiamine deficiency, however, appeared to cause a greater degree of slowing than could be accounted for by inanition and we are, therefore, inclined to attribute a significant part to thiamine deficiency specifically in the production of the bradycardia occurring in the deficient animals. Inactivity could not be regarded as a factor leading to slow heart rates in our animals for, unlike what is seen in certain cases of human beriberi, no neurological disturbances whatever occurred and the animals were able to get about quite normally. Only when thiamine deficiency was very severe and dyspnea and cyanosis were present, were the pigs reluctant to move about. When they were in this condition, forced exercise produced marked aggravation of the symptoms of heart failure and even caused death in some instances (1).

In several of our animals tachycardia was observed when cardiac failure had set in. In human cases of nutritional deficiency probably caused by thiamine deficiency, Weiss and Wilkins (6) described, among other signs, tachycardia, bounding arterial pulsation, arterial pistol sounds, and engorged veins, and called attention to the normal arterial pressure, increased venous pressure, increased velocity of blood flow and decreased peripheral utilization of arterial oxygen. The studies of Prados and Swank (26) revealed vascular dilatation in thiamine-deficient pigeons. Swank and Bessey (16) suggested that tachycardia in thiamine deficiency is caused by vasodilatation which is due to the local accumulation of intermediate products of carbohydrate metabolism.

With the view of Swank and Bessey that the manifestations of cardiac failure in thiamine deficiency are due to at least two factors, namely vasodilatation and the effect of thiamine deficiency on the myocardium directly, we do not take issue. However, our observations in pigs cause us to regard their second factor as of primary importance. The evidence at hand suggests that thiamine deficiency impairs the function of the heart by reducing the energy available from the normal processes of carbohydrate metabolism in which thiamine plays a role. Bradycardia is perhaps a means for sparing the heart under the unfavorable metabolic conditions of thiamine deficiency. Tachycardia is probably the expression of a decompensated heart and also, perhaps, the consequence of vasodilatation and reduced periph-

eral resistance. With vasodilatation serous effusions develop and all the signs of cardiac failure appear.

Attempts to demonstrate that the metabolites which accumulate in thiamine deficiency are the cause of the cardiac manifestations associated with such a deficiency have, in fact, failed. In normal and in vitamin B_1-deficient rats single and repeated injections were made by Haynes and Weiss (27) of sodium pyruvate, pyruvic acid, sodium lactate, lactic acid, sodium bicarbonate, ammonium chloride, methyl glyoxal, glyceraldehyde, glucose, and sodium adenylate. Although certain effects were observed and the vitamin B_1-deficient animals appeared somewhat more sensitive than normal animals to large doses of pyruvate, the results were not regarded as physiologically significant because of the large quantities which were required to produce these effects. In our own studies it is to be noted that, although the quantity of pyruvic acid in the blood of thiamine-deficient pigs was high at the time when pronounced electrocardiographic changes were found detectable abnormalities were noted even when the blood pyruvic acid was normal.

The bradycardia, the prolongation of the PR interval and the acceleration of the heart rate following injection of atropine observed in two of our animals, suggest vagal overaction. In rice-fed pigeons Carter and Drury (15) noted bradycardia and heart-block. These effects were relieved by section of the vagus nerves or by paralysis of vagal tone with atropine; they developed even in a pigeon whose cerebrum had first been removed; and a similar degree of vagus stimulation by pilocarpine was produced in normal and in rice-fed pigeons whose vagi had been sectioned. Carter and Drury concluded, therefore, that the heart block was the result of over-action of the vagal centers. In rats, Weiss et al. (20) found that atropine or vagus section had little effect on the heart rate in vitamin B_1-deficient animals, although in man Weiss (6) thought the cardiac changes in vitamin B_1 deficiency were in part due to changes in the vagus system. McEachern and Brophy (28) measured *in vitro* the beats of the isolated auricles of thiamine-deficient rats. They found that the bradycardia observed *in vivo* disappeared in the Dale bath and concluded that the bradycardia was of neurogenic origin. We have found that the bradycardia of inanition, like that of thiamine deficiency, is relieved by the administration of atropine.

The extreme sensitivity of the heart to lack of thiamine which these studies reveal deserves consideration. The obvious explanation is that continuous activity of heart muscle is necessary for life whereas this is not true to the same degree in the case of the skeletal muscles. The muscles concerned in respiration, however, must be active at all times also, yet necrosis in these muscles has not been observed in thiamine deficiency. The effort of the muscular activity may be a factor. None of these explanations, however, seems to be entirely satisfactory. Seeking another cause for the relatively great requirement of the heart for thiamine, it is to be noted that lactic acid has been found to be removed from the coronary arterial blood in quantities which suggest its utilization as the carbohydrate fuel of the heart (29). If this is true and if this lactic acid is derived from pyruvic acid, the question is not so difficult to answer for there is much evidence to show that the metabolism of pyruvic acid involves the use of thiamine diphosphate (cocarboxylase) (30).

SUMMARY

Thiamine deficiency is associated with pronounced electrocardiographic changes in the pig. These include bradycardia and prolonged PR interval as well as second degree auriculo-ventricular block, abnormalities in the P waves, inversion of T_4, nodal and ventricular premature beats, auriculo-ventricular dissociation, complete block with ectopic ventricular rhythm and auricular fibrillation.

Inanition alone may cause bradycardia but thiamine deficiency appeared to cause a greater degree of slowing than could be accounted for by inanition. It is concluded that in the thiamine-deficient pig bradycardia is attributable to the vitamin deficiency rather than, or in addition to inanition. Evidence is presented which indicates that the bradycardia as well as certain other signs are the result of vagal overaction.

The electrocardiographic changes are probably the expression of the disturbance in metabolism which is caused by a lack of thiamine.

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THE PLASMA PROTEINS IN DISSEMINATED LUPUS ERYTHEMATOSUS[1]

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Three quarters of a century have elapsed since the systemic nature of disseminated lupus erythematosus was first recognized (1); nevertheless few diseases remain more obscure than this process. This is due in part to its rarity which makes diagnosis difficult and early recognition almost impossible, and affords little clinical material for investigation. In a period of fifteen years the authors have been able to study the clinical course in only thirty patients. The organs of thirteen of these have been examined post-mortem, and there has been a striking disproportion between the extensiveness of the clinical manifestations and the paucity of histological lesions. Although during life the disease process has suggested a chronic infection, attempts at autopsy to secure evidence for an infectious etiology have been unsuccessful. The lack of inflammatory lesions in the fixed tissues and the failure to detect any causative agent suggested that perhaps the disease process was primarily one of altered physiology which could best be studied in vivo. In making observations on the lupus subject during life we have been impressed with the constant occurrence of hyperglobulinemia in this disease. It is the purpose of this paper to present these findings on the changes in the serum proteins found in the lupus subject and to suggest the possibility that there may be an association between hypergammaglobulinemia and the nutritional disturbances observed in disseminated lupus erythematosus.

CLINICAL MANIFESTATIONS

The thirty patients in the present series were similar to those described by other observers (2) (3) (4) (5). The clinical findings are presented in Table I.

[1] The work reported in this communication was carried out under the W. K. Kellogg Foundation Fund.

Thirteen of this group of thirty patients were of the Hebrew race. The ages varied from six to forty-eight; seven were under fourteen;

TABLE I

Clinical Manifestations Observed in Thirty Cases of Lupus

	NUMBER OF PATIENTS	NUMBER POSITIVE
Family history of rheumatic fever	30	1
Past history of rheumatic fever	30	1
Clinical manifestations		
1. Fever without leucocytosis	30	30
2. Anemia	30	30
3. Lupus on face—transient	30	25
4. Arthritis—migratory evanescent	30	23
5. Subacute nephritis	30	21
6. Heart muscle, change by EKG	30	18
8. Petechial hemorrhages	30	16
8. Characteristic fingertip changes	30	15
9. Edema—localized and evanescent	30	15
10. Transudation—body cavities	30	12
11. Excoriation of mucous membranes	30	12
12. Emaciation	30	10
13. Decubital ulcers	30	10
14. Retinitis	30	7
15. Lupus on palate	30	7
16. Amenorrhea	30	7
17. Purpura	30	7
18. Photosensitivity	30	7
19. General lymphadenopathy and splenomegaly	30	7
20. Chorea	30	5
21. Alopecia*	30	5
Laboratory findings		
1. Elevated blood sedimentation rate	20	20
2. A/G ratio reversal	17	17
3. Low Ca blood level	6	6
4. Low vit. C blood level	6	6
5. Wasserman positive or anti-complementary†	30	11
6. Kline positive	30	13

* Most patients showed some loss of hair.

† In early cases of this series only a single Wasserman test was made.

the majority were between fifteen and thirty. Except for one adult and three children all were female. The onset of symptoms occurred in the summer or early fall months and the disease in all but two

patients terminated fatally in from three to eighteen months. That there were widespread changes of small blood vessels was suggested by lesions in the finger tips, petechial hemorrhages, retinitis, nephritis, and purpura. Involvement of the serous membranes or the body cavities was manifested by pleural, pericardial, and abdominal effusions. The profound anemia and the lymph node and splenic enlargement indicated disturbances of the hematopoietic system. Electrocardiographic changes and intense muscle pains in several patients were indicative of muscle fiber lesions. Striking photosensitivity in the absence of porphyrinuria, changes in the character of the hair to a degree of complete alopecia, amenorrhea, and transitory nutritional disturbances were observed. Only the changes in the finger tips were found to be pathognomonic. They are illustrated in Plate I. Every clinical manifestation was found to be spontaneously reversible. Remissions and relapses were characteristic; however, in all but two of these patients the underlying process was relentlessly progressive. One of these exceptions has remained in a clinically quiescent state for four years. The other died of pneumococcus endocarditis about two years after recovery from the lupus process.

Although some of these patients manifested signs of chronic bronchitis early in their disease and although most of them had a fatal terminal infection, the clinical phenomena of the lupus process were not those customarily observed in sepsis. The lesions of the mucous membranes of the mouth and the exposed parts of the skin, like those of pellagra, appeared photosensitive. The aplastic type of anemia, frequently accompanied by achlorhydria, the alteration of vascular permeability with massive effusions of a low specific gravity fluid, the changes in the character of the hair leading in five patients to alopecia, all suggested defects in cellular nutrition. These disturbances were manifested early in the course of the disease and were not modified by high caloric diets reinforced with the known vitamins. Some of the laboratory findings were also indicative of metabolic disturbances. Blood calcium levels were low; blood pantothenic acid values were markedly increased in the five patients tested; blood vitamin C levels were low even during the afebrile phases. Furthermore in these individuals large oral doses of vitamin C and intravenous doses of ascorbic acid

failed to raise the blood level to normal values.[2] Since no increase in excretion of vitamin C was noted it appeared that either ascorbic acid was destroyed or retained in tissues.

ANATOMICAL FINDINGS

Thirteen of these subjects were examined post-mortem and careful histological studies revealed little in most instances. The microscopic findings were limited in some cases to those commonly found in terminal infections. Five patients had verrucous endocarditis as described by Libman and Sachs (6). Thickening of the pericardium, pleura, and capsules of spleen and liver, and bronchopneumonia were also noted in eleven cases. In several patients the pathologic basis for the diagnosis was slight. Only two patients had extensive vascular lesions. In general the findings were similar to those described in detail by others (7). The following organs were involved in one or more patients:

Endocardium—Libman-Sachs verrucae (5 cases).

Myocardium—Focal necroses situated about small blood vessels with necrosis in the wall of the vessel, fibrinous exudate and collections of mononuclear cells (8 cases).

Kidneys—"Wire loop" lesions (8) of the glomerular tufts with thickening of the basement membrane (9 cases).

Skeletal muscle—Focal lesions (in the absence of vascular changes) with marked irregularity of fibers, hyaline necrosis of individual fibers, and occasional regenerating cells (2 cases).

Lymph nodes—Large patches of necrosis with much nuclear fragmentation (3 cases).

Spleen—Scarring of the central portion of the Malpighian bodies. (2 cases). Accessory spleen (4 cases).

Bone marrow—Aplasia (2 cases).

[2] In a report of vitamin C saturation levels in various pathological conditions, Finkle (*J. Clin. Invest.*, **16:** 587, 1937) studied five cases of lupus erythematosus. He observed that the excretion of vitamin C was only 30 per cent of normal and that following the intravenous administration of 100 mgm. of vitamin C there was no rise in urinary output of this vitamin. He concluded that: "The persistently low excretion level of vitamin C in acute lupus erythematosus differs from that found in any other condition studied."

Liver—Focal central necrosis and excess fat (6 cases).

Stomach—Atrophy of the gastric mucosa (2 cases).

Adrenal—Diffuse fibrosis of the adrenal cortex and lipid depletion (3 cases).

Testis—Atrophy and absence of spermatogenesis (1 case).

Pituitary—Focal necroses (1 case).

Skin—Marked edema of the corium and papillary bodies and occasional perivascular infiltrations (2 cases).

Lungs—In addition to terminal infections there occurred chronic changes characterized by marked proliferation of the epithelium and occlusion of alveoli with fibrous plugs (10 cases).

Arteries—The arterial lesions resembled, but were not identical with, those seen in rheumatic fever. The primary change seemed to be the exudation of coagulable material into the subendothelial tissue over a limited area, Plate II, C. Part of this material, frequently broken into clumps, stained blue with hematoxylin but was obviously not calcium. In many vessels the internal elastica was ruptured. The endothelial cells were swollen or degenerated and nuclear fragments were seen in the fibrinoid material. In other vessels, reactive proliferation of spindle cells had occurred which formed plugs completely filling the original lumen. About the adventitia of other vessels a mild type of inflammatory reaction was present, composed chiefly of plasma cells. There was another type of perivascular change, which appeared of longer standing. This took the form of concentric laminae of elongated connective tissue cells and fibers as described by Klemperer, Plate III, H. These different stages in the development of vascular lesions are shown in Plates II and III.

The anatomical changes were disproportionately slight as compared with the severity of the clinical symptoms. All but one of these patients died while in a clinically active state, and yet no uniform changes were found. Lesions of rheumatic fever and tuberculosis were entirely absent. The vascular lesions, when present, seemed to begin with the exudation of coagulable material into the wall of the vessel, leading to progressive occlusion and finally thrombosis. Inflammatory reaction except in a few vascular lesions was conspicuously lacking.

Changes in the pericardium, pleura and peritoneum were similar to those seen in non-tuberculous polyserositis. Since histological studies failed to throw any light on the nature of the lupus process, it appeared that structural changes which accompanied the widespread clinical phenomena were transient, or that the symptoms were due to alterations in cell physiology which could be analyzed only during life.

FRACTIONATION OF THE SERUM PROTEINS

One constant clinical finding was acceleration of the erythrocyte sedimentation rate even when corrected for anemia. The sedimentation rates in these patients were usually more rapid than in acute infections, and were comparable to those observed in multiple myelomata and lymphogranuloma venereum. Investigation showed that this was attributable to marked increases in serum globulins. Fractionation of the serum globulins of fifteen patients having lupus was carried out according to Howe's method as described by Gutman et al (9). The results are presented in Table II.

It is seen in Table II that these fifteen subjects, females between the ages of six and thirty-six years, all showed an inversion of the albumin-globulin ratio. The total protein was in most instances within the normal range. Each albumin determination was below normal (median 3.1). Each patient developed a total globulin above normal (median 3.8) and each euglobulin value was found to be markedly elevated (median 1.0).

Repeated fractionations of the serum proteins during the course of this disease showed (a) that the inversion of the albumin-globulin ratio followed and did not precede the development of the symptoms of the lupus process (Case 5); (b) that the progress of the disease was accompanied by an increase in euglobulin (Cases 4 and 11); (c) that return to a quiescent state was attended by a decrease in serum globulin (Cases 14 and 15); (d) that four years after recovery the albumin-globulin ratio was 1 to 1, the total globulin 3.8, the euglobulin 0.7 (Case 15) and (e) that the patient who at autopsy had widespread arterial lesions with exudation of coagulable material into the subendothelial tissues (Case 13, Plate II) showed the most striking hyperglobulinemia, total globulin 4.8 and euglobulin 2.1 grams per 100 cc. of serum.

Two other methods were employed in the study of the globulins. Kendall (9) applied the precipitation technique to sera from three of

TABLE II

Partition of Serum Protein in Cases of Lupus by Howe's Method

(Results expressed in grams per 100 cc. serum)

NUMBER	SEX	AGE	TOTAL PROTEIN	ALBUMIN	TOTAL GLOBULINS	EUGLOBULIN
1	F	33	7.1	2.4	4.7	1.5
2	F	23	7.1	3.4	3.7	1.3
3	F	36	6.2	2.6	3.6	0.8
			6.2	2.5	3.7	0.9
4	F	21	6.9	3.4	3.5	0.8
			6.8	3.1	3.7	1.0
			7.9	3.6	4.3	1.5
5	F	12	5.7	3.3	2.4	
			5.1	2.8	2.4	
			4.2	2.1	2.1	
			6.2	2.4	3.8	
			6.2	2.4	3.8	
			6.6	3.0	3.7	
			7.1	3.4	3.7	
6	F	22	8.2	3.7	4.5	1.0
7	F	15	6.4	3.2	3.2	1.2
			6.6	3.0	3.6	1.0
			6.1	2.8	3.3	0.8
			5.8	2.6	3.2	
			6.5	3.1	3.4	0.9
			7.4	3.4	4.0	1.3
			6.1	2.2	3.9	1.2
8	F	17	7.2	2.9	4.3	1.7
			8.3	3.0	5.3	1.9
9	F	15	7.3	3.7	3.6	1.1
			6.6	3.2	3.4	
10	F	29	7.0	2.5	4.5	
			6.4	2.4	4.0	

TABLE II—*Continued*

NUMBER	SEX	AGE	TOTAL PROTEIN	ALBUMIN	TOTAL GLOBULINS	EUGLOBULIN
11	F	22	7.6	3.8	3.8	0.7
			7.3	3.7	3.7	0.7
			7.0	3.2	3.8	0.9
			6.5	2.5	4.0	0.9
			8.7	3.0	5.7	2.2
			7.9	2.8	5.1	1.4
			6.9	2.3	4.6	2.1
12	F	6	6.5	2.9	3.6	
			7.9	3.6	4.3	
			8.0	3.0	5.0	
			5.9	2.8	3.1	
			7.7	3.6	4.1	0.7
13	F	24	6.3	1.5	4.8	2.1
14	F	21	8.0	3.4	4.6	1.9
			7.3	2.9	4.4	
			8.0	4.1	3.9	1.0
			8.6	4.4	4.2	1.3
			7.0	3.6	3.4	1.0
			7.9	3.8	4.1	0.7
			8.2	4.3	3.9	1.0
			7.6	3.9	3.5	0.8
			7.7	3.9	3.8	1.0
15	F	23	7.5	3.1	4.4	
			7.5	3.4	4.1	0.9
			6.0	2.4	3.6	1.3
			6.8	2.3	4.5	1.2
			7.5	3.7	3.8	0.8
			7.9	3.9	4.0	
			7.8	4.0	3.8	0.7
			7.7	3.9	3.8	0.7
			8.2	4.2	4.0	1.0
Maximum			8.7	4.4	5.7	2.2
Minimum			4.2	1.5	2.1	0.7
Mean			7.0	3.1	3.9	1.1
Median			7.1	3.1	3.8	1.0

these patients. All showed an increase in the Tiselius gamma fraction. Electrophoretic analyses were made on plasma from two pa-

tients; one, throughout the course of the disease activity, the other in a quiescent state three years after clinical recovery. The case histories are briefly as follows:

Case #12. This six year old girl was of Russian Hebrew parents. Her birth and early childhood were normal. At the age of six she lost appetite, vomited, and developed fever.

The patient entered the hospital in February, 1942, remaining until her death in January, 1943. During the first few months of her admission she had a Pel-Epstein type of fever. This was followed by a remission which extended from June 3rd until July 23rd. Following this, the temperature was irregularly elevated until exitus. During the remission of fever she was symptomatically improved. Her course was progressively worse during the last six months of life.

While under observation, she manifested the classical symptoms and signs of lupus disease—prolonged fever without leucocytosis, profound anemia, a transient butterfly lesion on the face, joint pains, occasional albuminuria, EKG changes indicating heart muscle damage, characteristic finger tip changes with atrophy and necrosis of the dermis around the margin of the finger nails and swelling and hemorrhages of the finger tips, transitory localized areas of edema, excoriations of the lips and nares, lupus lesions in the hard palate, marked emaciation, generalized lymphadenopathy, enlargement of the spleen, moderate alopecia.

Laboratory findings included—marked and constant elevation of the blood sedimentation rate, reversal of the albumin-globulin ratio, with marked increase in the eu-and pseudo-globulin fractions, positive Wassermann and Kline reactions, and low vitamin C blood levels.

The autopsy findings (Babies Hospital, Case #6773) were: *bronchiectasis of right and left lower lobes; polyserositis of right and left pleura, perihepatitis and perisplenitis; thromboses of veins within the spleen with hemorrhagic infarcts; hyperplasia of the lymph glands with hemorrhage in the glands; hepatomegaly with central necrosis of the liver.*

Histological examination of the liver sections (by Dr. Dorothy Andersen) is reported as follows:

"In the portal areas there is slight increase in fibrous tissue and a mild infiltration of phagocytes and lymphocytes, and there is a small amount of bile-duct proliferation in a few portal areas. The liver cells near the portal areas are of normal size and contain little or no fat. The nuclei vary somewhat in depth of stain and in size. The cytoplasm has a very finely granular ground glass appearance. This description holds for the third of each lobule nearest the portal area. In the middle zone the liver cells are pale, contain many fat droplets of all sizes and the sinuses are somewhat congested. In some of the central areas there appears to be a fairly wide space between the walls of adjacent sinuses. These walls are somewhat thickened and look as though they were more rigid than usual. In the space between them there are small round cells, some of which appear to have arisen

as liver cells. Others more closely resemble phagocytes and many cannot be identified surely. These areas are not sharply demarcated and do not involve every central area. Often they occupy only a part of a given central area. In some instances the liver columns in the central areas are represented by irregular masses deeply eosinophilic amorphous material which resembles coagulated protein. Such an appearance is usually found near the center of a larger area and the peripheral portion is composed of liver cell columns with shrunken nuclei as described above. Beneath the capsule there are several such areas involving the entire central part of a lobule in a sort of coagulation necrosis. The outer portion of such an area is marked by an infiltration of phagocytes and polymorphs with many fragmented nuclei. Such large areas of necrosis are not found deep in the liver tissue. The capsule is somewhat thickened and bears a rather abundant fibrinous exudate which shows partial organization but no purulent reaction. In some of the denser massess of fibrin there is a deposition of bluish granular material.

Central necrosis of the liver was extensive in this patient. Of especial interest were the areas in which liver columns appeared to be replaced by masses of amorphous eosinophilic material resembling coagulated protein. Low and high power photomicrographs are shown in Plate IV.

Case #15. This patient was a 23 year old Scotch-American housewife, the mother of two children. Except for a severe cervical adenitis in childhood she had enjoyed good health until the summer of 1937, when she developed pain in her shoulder and abdomen.

She was first admitted to the hospital in November, 1937 because of severe abdominal pain and was found to have polyserositis with pericarditis, pleurisy and fluid in the abdomen. She was readmitted three times during 1938 and 1939 with irregular fever, pleural effusions, and severe furunculosis.

During these four admissions she presented many of the classical findings of this disease—prolonged fever without leucocytosis, anemia, joint pains, polyserositis, EKG changes indicating myocardial damage, occasional albuminuria, lymphadenopathy, mild alopecia and transitory localized areas of edema. The blood sedimentation rate was constantly elevated; there was a reversal of the albumin-globulin ratio, and the blood calcium values were low. The chest fluid was found to be negative for tubercle bacillus on smear and guinea pig innoculation.

In November, 1939, she was emaciated, febrile, had severe blepharitis, aural discharge, draining axillary carbuncle, generalized furunculosis, profuse cervical discharge, draining abscess of the ankle, sharp pains in the chest and abdomen, a severe weeping rash under each mammary gland, a cough productive of grayish sputum, and migratory joint pains. In addition she was amenorrheic and complained of severe headaches and hot flashes.

At this time she was given estrogen (progynon-B) 10,000 units weekly. This

was continued for six months when the dosage was changed to 20,000 units every two weeks for six months. She was then given stilbestrol, 1 mg. daily for four months. After this estrogen therapy was stopped.

With the institution of estrogen therapy, there was a rapid disappearance of headaches and hot flashes. The appetite improved and malaise disappeared. The

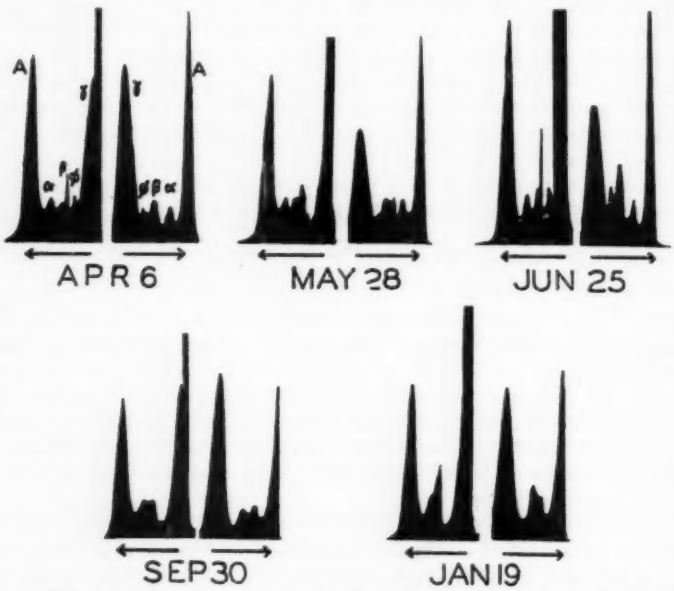

FIG. 1. ELECTROPHORETIC PATTERNS ON PLASMA FROM CASE #12 TAKEN ON THE DATES INDICATED

Descending pattern migrating left; ascending, right. Non-converging streaks over the descending gamma globulin patterns caused by opaque precipitate. Slightly lower gamma globulin on May 28th may be associated with clinical improvement noted in June.

draining abscess of the ankle healed and she maintained a normal temperature. At the end of three months she had gained about six pounds and looked a great deal improved. The heart shadow had become smaller. After six months of estrogen therapy she began to menstruate and continued to do so. The blepharitis and eczematoid lesions below the breasts slowly disappeared.

Slow but progressive improvement of all symptoms and signs continued during 1940. The patient has enjoyed good health during the

period of 1941–1943. However, the blood sedimentation rate remains elevated and the serum globulin fraction is increased. It is of interest that the only other patient to make a clinical recovery was treated similarly with estrogen.

ELECTROPHORETIC ANALYSIS OF PLASMA FROM CASES OF LUPUS ERYTHEMATOSUS

Samples of plasma from these two patients were diluted with three parts of a 0.02 M sodium phosphate buffer containing 0.15 M NaCl at pH 7.4 and analyzed in the Tiselius electrophoresis apparatus (11). The Toepler Schlieren optical method as modified by Longsworth was used (12). The electrophoretic patterns on samples of plasma drawn

TABLE III

Case # 12. Electrophoretic Data Deduced from Patterns of Fig. 1

DATE PLASMA WAS TAKEN	COMPOSITION DATA												MOBILITIES × 10					
	A/G	α/A	β_1/A	β_2/A	ϕ/A	γ/A	A%	α%	β_1%	β_2%	ϕ%	γ%	A	α	β_1	β_2	φ	γ
4/ 6/42	.60	.18	.10	.15	.11	1.24	36.1	6.5	3.7	5.2	3.8	44.7	5.1	3.5	2.6	2.2	1.6	0.3
5/28/42	.82	.18	.17	.16	.06	.71	44.0	8.0	7.3	6.8	2.6	31.3	4.9	3.4	2.7	2.2	1.0	0.3
6/25/42	1.04	.15	.10	.25	.16	.46	47.2	6.9	4.9	11.8	7.3	21.9	4.7	3.1	2.3	2.0	1.3	0.3
9/30/42	.52	.26	.19	—	—	1.22	37.4	9.6	7.2	—	—	45.8	5.5	3.6	2.9	—	—	0.5
1/19/43	.77	—	.19	.28	—	.83	43.6	—	8.1	12.2	—	.83	5.3	—	2.9	1.3	—	0.7

from Patient # 12 on the dates indicated are shown in Fig. 1, and the electrophoretic composition of each sample is given in Table III. All of the patterns exhibit an excessive amount of gamma globulin, giving an abnormally low A/G ratio. The other globulin fractions are approximately normal (13). A portion of the gamma globluin was isolated and the sedimentation rate was determined in the ultra centrifuge (14). The sedimentation constant was found to have a value of about 7S, the same as that for normal gamma globluin.

Several other conditions have been observed in which such abnormally large amounts of gamma globulin occur. One is in hyperimmune antipneumococcus horse sera (seen in Fig. 2) (15). Others include certain cases of multiple myeloma (16) (9), liver disease, lymphogranulomata venereum and sarcoid. In the first instance, most of the gamma globulin is antibody which may be absorbed by specific poly-

saccharides leaving a normal pattern. In the second case, the excess gamma globulin may either be an antibody to Bence Jones protein, which has been shown to be present in the blood of certain cases of multiple myeloma (17), or may be due to the pathological condition existing at the source of the globulin.

In Fig. 3 is shown the electrophoretic pattern, and in Table IV is given the composition data for plasmas from Case #15 three years after all clinical symptoms of lupus had disappeared.

The gamma globulin here also is abnormally high and in the descending limb of the electrophoresis cell, where the other proteins have

FIG. 2. ELECTROPHORETIC PATTERN OF HYPERIMMUNE ANTIPNEUMOCOCCAL HORSE SERUM

moved away, there is an opaque precipitate. This opaque layer is also observed in varying degrees in all descending patterns in Fig. 1, making the gamma peak appear infinite in height. In phosphate buffer at pH 7.4 this phenomenon has been observed for most sera or plasma giving a positive Wassermann reaction, but to our knowledge not in sera with negative serological reactions.

ANALYSIS OF FALSE POSITIVE WASSERMANN REACTIONS

None of these patients had clinical or anatomical evidence of syphilis and some were known to have negative serological reactions prior to the onset of their disease. Altogether eleven patients had positive Wassermann and thirteen had positive Kline tests. The results of the Wassermann reactions fluctuated from anti-complementary to positive

or negative and the Kline tests from ++++ to 0, irrespective of the presence or absence of pyrexia. Eight patients had ++++ or anti-complementary Wassermann reactions and all of these sera gave ++++ Kline tests. Three patients gave + to +++ Wassermann

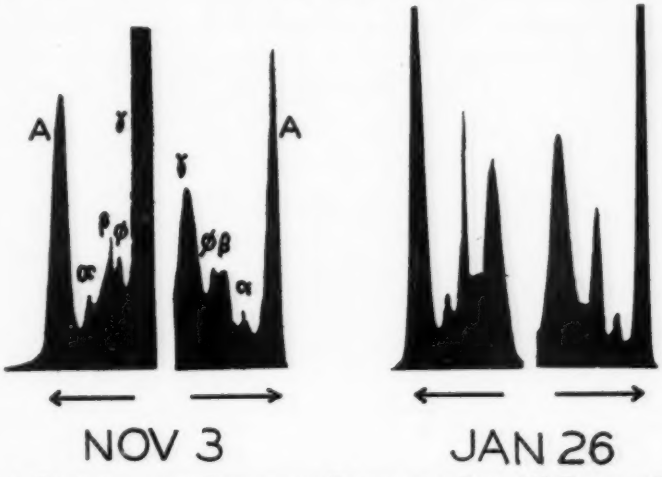

FIG. 3. ELECTROPHORETIC PATTERNS ON PLASMA FROM CASE #15, TAKEN AT TWO DIFFERENT DATES, BOTH AFTER CLINICAL RECOVERY
Left, descending pattern; right, ascending

TABLE IV

Case #15. Electrophoretic Data Deduced from Patterns of Fig. 3

DATE PLASMA WAS TAKEN	COMPOSITION DATA													MOBILITIES × 10					
	A/G	α/A	β_1/A	β_2/A	φ/A	γ/A	A%	α%	β_1%	β_2%	φ%	γ%	A	α	β_1	β_2	φ	γ
11/ 3/42	.75	.15	.38	—	.21	.58	43.2	6.6	.87	.76	.89	.25	5.3	3.5	2.3	—	1.8	0.3
1/26/43	.76	.16	.43	—	—	.73	43.2	6.8	18.5	—	—	31.5	5.1	3.6	2.6	—	—	0.8

reactions with positive Kline tests. In two patients the Kline tests were weakly positive and the Wassermann reactions negative. The following studies were made to differentiate the serological reactions of secondary syphilis from the false positives occurring in lupus erythematosus.

Variability of False Positive Reactions in Lupus: Unlike the sero-

logical reactions of active syphilis, there were marked fluctuations in the Wassermann and Kline tests from week to week. This variability is seen in Table V.

It is seen in Table V that in the three day period between 5–8 and 5–11, the false positive reactions entirely disappeared and that between 5–21 and 5–28, the reactions reappeared in the serum of patient #13. Clinical improvement appeared at the time of the negative reactions.

Those lupus sera giving positive reactions were only positive to a titer of 1:5.

TABLE V

Wassermann and Kline Reactions of Patient #12 over a Period of Seven Months

DATE	WASSERMANN		KLINE
	Alc. antigen	Chol. antigen	
4/9	AC	AC	++++
4/16	AC	AC	++++
4/30	AC	++++	++++
5/8	AC	++++	++++
5/11	Negative	Negative	Negative
5/21	Negative	Negative	Negative
5/28	AC	AC	++++
6/22	Negative	+++	++
9/25	AC	AC	++++
11/24	AC	AC	++++

Absorption Experiments: Samples of sera from these two diseases were absorbed with four phospholipids; (1) cephalin, (2) lecithin, (3) inositol phosphatide and (4) Kline antigen. The absorptions were done as follows:—An equal volume of serum and phospholipid were mixed and allowed to stand overnight at room temperature. The mixture was then centrifuged; the supernatant was removed, inactivated at 56 degrees C. for 30 minutes and then tested with the Wassermann and Kline antigens.

The results were as follows: Absorption with cephalin, lecithin and inositol phosphatide had no effect on the sera. A single absorption with the Kline antigen had no effect on the syphilitic serum and completely removed the reactivity of the lupus serum to the Kline antigen. However by repeated absorptions with the Kline antigen the antibody

TABLE VI

The Effect of Temperature on the Wassermann Reactivity of Lupus and Syphilitic Sera

	LUPUS SERUM +++								SYPHILITIC SERUM ++++					
	Serum dilution													
	*Control	1 in 1	1 in 2	1 in 10	1 in 20	1 in 40	1 in 60	Control	1 in 1	1 in 2	1 in 10	1 in 20	1 in 40	1 in 160
	Quantity in cc.													
	.04	.02	.02	.02	.02	.02	.02	.04	.02	.02	.02	.02	.02	.02
Unfortified alcoholic antigen 1 in 25; antigen control 0.5 and 0.2 cc. negative														
Temp. (C.) at which serum was inactivated														
56°—30 min.	+++	++	0	0	0	0	0	0	++++	++++	++	+	0	0
62°— 5 min.	+++	++	0	0	0	0	0	0	++++	+++	0	0	0	0
62°—30 min.	+++	+++	++	++	++	+	0	0	++++	+++	0	0	0	0
62°—60 min.	+++	+++	+++	+	+	+	+	0	++++	+++	0	0	0	0
Cholesterin antigen 1 in 45; antigen control 0.1 and 0.2 cc. negative														
Temp. (C.) at which serum was inactivated														
56°—30 min.	++++	+	0	0	0	0	0	0	++	+	0	0	0	0
62°— 5 min.	++++	++	0	0	0	0	0	0	0	0	0	0	0	0
62°—30 min.	++++	++++	++	0	0	0	0	0	0	0	0	0	0	0
62°—60 min.	++++	++++	++	+	+	0	0	0	0	0	0	0	0	0

* Lupus serum anticomplementary only when undiluted.

of the syphilitic serum was also removable. This difference in the reactivity of the two sera indicates that the antibody present in the lupus cases is much weaker than in syphilis.

The Effect of Increased Temperature: The Wassermann reactivity of sera from these two diseases was tested after aliquots had been heated as high as 62 degrees C. and for intervals of 5 to 60 minutes. The results are presented in Table VI.

It is seen in Table VI that a ++++ syphilitic serum lost reactivity on heating to 62 degrees for as little as 5 minutes. The lupus serum, which originally gave a weaker reaction than the syphilitic serum, became more strongly positive as the temperature was raised, and gave the strongest reactions when raised to 62 degrees C. for the longest period tested (60 min.). This suggested that in addition to the differences in quantity of antibody there was a qualitative difference between the antibodies of syphilitic and lupus subjects.

Isolation of the Wassermann Antibody: Globulin fractions separated electrophoretically were tested for activity with the Wassermann and Kline antigens. The nitrogen content of the samples to be tested was determined and protein solutions of approximately equal nitrogen values were used. The antibody was found to be mostly in the gamma fraction and partly in the $beta_2$ fraction, indicating that it migrated with a mobility between that of beta and gamma globulin.

DISCUSSION

Hyperglobulinemia is a constant characteristic of the lupus state. Electrophoretic analysis shows that this increase in globulin is chiefly in the gamma fraction. This fraction or some component of it reacts with the Wassermann and Kline antigens. The factor initiating the production of this reactive protein is obscure. Although this gamma globulin is indistinguishable from the antibody of syphilis, lupus subjects do not have syphilis. That this protein is produced as a result of a chronic infection is not substantiated by the anatomical findings at autopsy unless the mild bacterial infections of the bronchial tree are significant. Another possibility is that tissue damage occurs following a simple bacterial infection and results in the production of an auto-antigen in a peculiar host. Schwentker and Rivers (18) have produced complement-fixing auto-antibodies in the rabbit. During

the course of infection with yellow fever virus auto-precipitins have been demonstrated by Hughes (19). That iso-immunization can give rise to the disease of the newborn during pregnancy has been clearly demonstrated by Levine and his co-workers (20). Irrespective of the cause, hypergammaglobulinemia occurs early in the lupus process and is constantly associated with this state.

Possible effects of a high concentration of gamma globulin in the circulating blood stream are unknown. That the arterial and renal lesions of the lupus state may be referable to the deposition of coagulable material resembling serum protein has been pointed out, and it seems likely that such physical changes may give rise to the vascular phenomena observed during life. In six patients extensive liver damage was found at autopsy and this impairment may explain some of the metabolic disturbances which accompany the lupus process.

Furthermore, it has been shown that the gamma globulin in the sera of lupus patients has a marked in vitro avidity for phospholipids of the Wassermann and Kline antigens and that this reactivity increases with denaturation of the protein by heat. Whether similar reactions occur in vivo is unknown. If they do, this may indicate a profound disturbance in phospholipid metabolism and subsequent disturbances of cellular nutrition and metabolism.

This presentation raises more questions than it answers. Its purpose is achieved, however, if it reorients investigation of this disease away from a bacterial agent toward a peculiar host response in which clinical manifestations are associated with a high concentration of circulating gamma globulin. Disseminated lupus appears to be a disease of disturbed cellular metabolism, perhaps associated with the presence of this reactive protein.

SUMMARY

1. Hypergammaglobulinemia of unknown cause is a constant characteristic of disseminated lupus erythematosus.

2. In vitro this gamma globulin fraction reacts with certain phospholipids giving false positive Wassermann and Kline tests.

3. The suggestion is made that the vascular lesions and disturbances in nutrition observed in this disease may be associated with the presence of a high concentration of circulating gamma globulin.

The authors are deeply indebted to the Departments of Pathology of the Presbyterian and Babies Hospitals for the use of the autopsy material, to Dr. D. W. Woolley for the pantothenic acid assays, and to Mrs. Ethel Benedict Gutman for the protein fractionations.

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PLATE I

This colored drawing of the hand in the second month of the disease shows the hemorrhagic plaques on the palmar surfaces, the swollen, hemorrhagic but non-tender finger tips, and the eroded, purpuric lesions extending under the finger nails.

PLATE II

These eight photomicrographs show different stages in the development of lesions of small blood vessels from patient #13, Presbyterian Hospital autopsy #13748 performed by Dr. Edith Sproul.

A (upper left). Early renal lesion showing the accumulation of coagulable material in the glomerular loops.

B (upper right). Early arterial lesion (spinal cord) showing a large amount coagulable material beneath the endothelium.

C (lower left). A slightly more advanced arterial lesion (adventitia of aorta) also showing coagulable material beneath the endothelium.

D (lower right). A more advanced arterial lesion (pancreas) showing early organization.

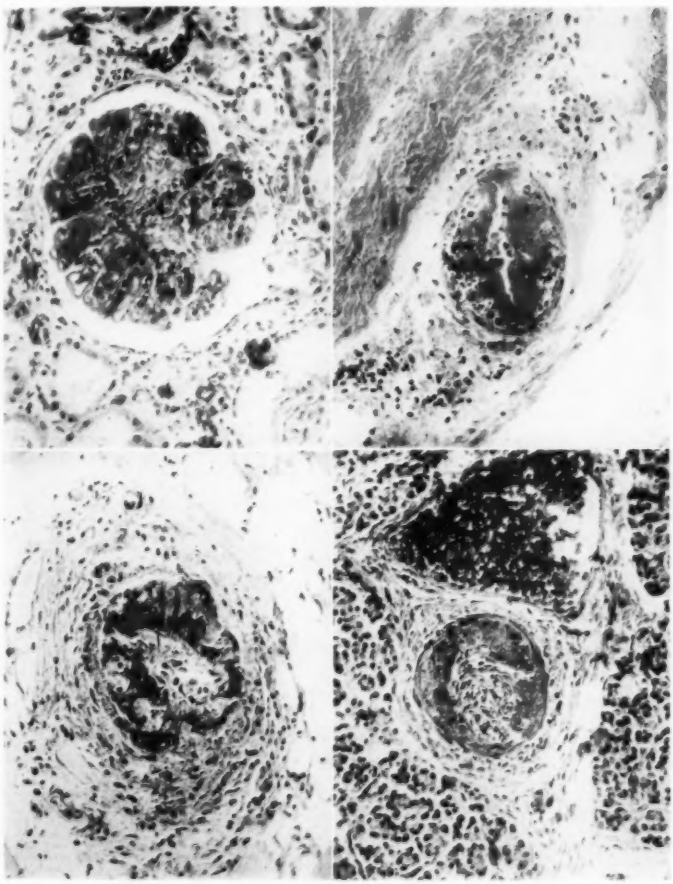


PLATE III

E (upper left), F (upper right), G (lower left). Arteries (pancreas, pectoral muscle and pancreas) show late stages of organization with occlusion of lumen.

H (lower right). Advanced arterial lesion (heart muscle) showing thick ring of sclerotic collagen fibers as described by Klemperer.

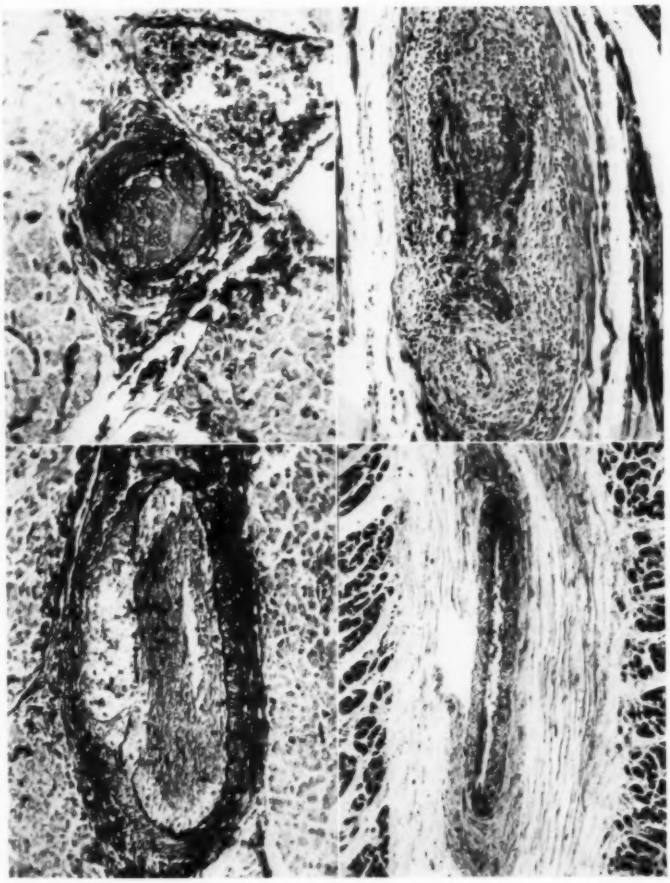
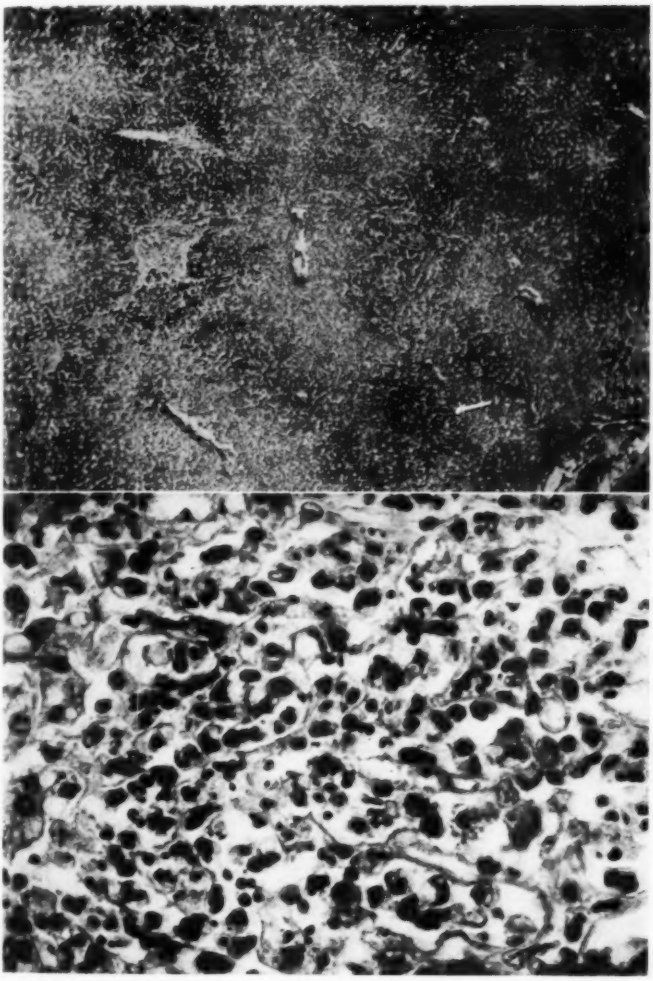


PLATE IV

Low and high power photomicrographs of the liver from Patient #12, Babies Hospital, autopsy #6773 performed by Dr. Dorothy Andersen showing central necrosis.



SCAPULECTOMY AND CLEIDECTOMY IN THE RAT

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Studies of alterations in morphology of the rat produced by amputating forelimbs have been carried out by Colton (1929, 1930) and of modifications in the activities of such animals by Pratt (1943a). These experiments were suggested by work done by Fuld (1901) and Knickmeyer (1895) (in Fuld 1901) some years before. The ability of the animal to compensate for the absence of the fore limb was evaluated, and anatomical modifications were studied. This method of approach opens some interesting avenues of investigation with regard to the pectoral appendage.

Two questions that arise deal with the importance to the animal of the scapula and clavicle. Excision of the scapula in the human has been performed on several occasions in order to remove tumors which were usually primary but in some cases were metastatic (Cairns, Cazin, McEwan, Ryerson). De Nancrède (1909) reviewed 65 cases of unilateral scapulectomy and 9 cases in which the other bones of the pectoral appendage were partially excised. These cases were selected as examples of primary malignant disease, treated by total excision. "Excellent functional result", reported in one instance, was obtained by suturing m. trapezius to m. deltoideus, following excision of the scapula (Ryerson). The patient was then able to move his forearm and hand "freely" through an arc of 20 degrees in any direction. In the other operations no discussion of the resultant function was given, but one concludes that function of the forearm and hand was good, while stability of the shoulder joint was lacking. Although the literature has been searched, no reference to congenital absence of the scapula was found.

Absence of the clavicle is more frequently reported in the literature, and is due to two primary factors. First, there is congenital absence of the clavicles, which is commonly associated with imperfect formation

[1] Henry Strong Denison Scholar 1942–43, 1943–44.

of the membrane bones of the skull and constitutes a syndrome known as cleido-cranial dysostosis. (Davis and Flecker, Eldridge, Hamilton, Kelley, McCurdy and Baer, Stahmann, Stern, Strauch). This is of especial interest, as the clavicle is a membrane bone, and the syndrome is a reflection of some anomaly in the osteogenesis of membrane bones in the individual. This is apparently carried as a dominant Mendelian character and has been shown to run through families (McCurdy and Baer). These individuals manage very well without clavicles, and are at times employed as the "India rubber man" in side show exhibits. As a rule, the sternal end of the bone is present, but in some cases no clavicle at all is demonstrable.

Second is a group of patients in which cleidectomy is performed for a variety of reasons. In 1910, Coley reported a series of cases of sarcoma of the clavicle and at that time summarized 63 cases from the literature. Since that time there have been several more cases reported (Campi and Maurano, Carson, Malatray and Bastien, Ricketts). Briggs (1939) reported on the use of a tibial bone graft to supply the deficiency left following resection of the middle third of the clavicle. In most instances, the deficit has been left untreated, and no great reduction in function has resulted, the patient being able to use the arm satisfactorily and with it to conduct a normal life. Such cases are relatively rare, and have great interest as these people are possessed of great flexibility and mobility of the shoulders.

In order to study these conditions experimentally, it was desirable to undertake the removal of scapulae and claviculae from very young white rats.

SCAPULECTOMY

Methods

For this purpose, litters of week-old laboratory rats were utilized, as in studies upon bipedal gait and experimental masseterectomy (Pratt 1943a, 1943b), and following proper preparation and anaesthetization with ether, the skin over and parallel to the spine of the scapula was incised. The extrinsic scapular muscles were divided, leaving the intrinsic scapular musculature intact and attached to the scapula, which was then removed. In sectioning the inferior rhomboid, about the angle of the scapula, bleeding was frequently encountered, especially in the larger rats that were occasionally used. The vertebral margin of the scapula was then elevated, m. subscapularis was dissected off the subscapular surface, and the

glenoid fossa was approached, exposing the head of the humerus by incising the joint capsule. The scapula was finally removed by dividing its ligamentous and muscular attachments, taking care to avoid the brachial plexus. The free appendage was left attached to the body wall by the clavicle, its muscular attachments, blood vessels and nerves, but without articulation with the scapula or connection with the intrinsic scapular musculature. In this operation, no profuse bleeding was encountered, except at the angle of the scapula, where occasionally a vessel was ligated. The wound was then rinsed with normal saline, and sutured. No case of postoperative infection was observed and recovery was rapid. In one instance, the animal climbed the wall of the cage within fifteen minutes of the last operation. To reduce mortality and to provide for the welfare of the animals, they were operated upon in two stages, removing one scapula at the first operation and the other scapula one week later.

Results

Activity. In activity, the scapulectomized animals cannot compete with either a normal rat or a cleidectomized animal. Their elbows protrude abnormally, due to rearrangement of the shoulder girdle (see plate 1). Lack of strength in the forelimb is particularly noticeable when they jump from a shelf to the floor of the cage. They jump as far out from the perch as is possible and land flat on their bellies, apparently endeavoring to get their hind legs in under them before they hit the floor. They never jump straight down, landing on their front legs, as they do not have sufficient forelimb support to break the fall by these members. In this respect they are like bipedal animals.

In all activities they are very cautious, as though uncertain or timid. This is well illustrated when they enter a new terrain, as they never jump into a new region without first testing it to make sure that it is solid, and that they can reach it without bringing undue strain upon the fore limbs.

After some time in a special cage, they learn to jump onto shelves like bipedal rats, but they are slower than bipeds in learning. They also learn to climb the sides of the cage, doing most of the work with the hind legs and less with the front legs. In most climbing, the front limbs seem to be used only for grasping the wire of the cage to prevent falling backward, while lifting of the body weight is done entirely with the hind legs.

Especially when standing on the hind legs, the elbows protrude characteristically, and the hands are crossed (see plate 1, nos. 2, 3, 4).

In turning, with forefeet touching the floor the fore limbs become crossed due to lack of control of their position.

The scapulectomized male has sufficient strength in the pectoral appendage to hold and impregnante the cleidectomized female. The scapulectomized female has no difficulty during gestation or caring for a litter of young.

In ordinary locomotion, the animal travels slowly, using all four feet, the front ones inefficiently. When higher speed is desired, the animal hops, kangaroo-like, in the same manner as the biped.

Anatomical Alterations. Changes in the anatomy of these animals are particularly striking in the case of the scapulectomized individuals. These rats were X-rayed during life, and it was seen that the level of the head of the humerus had moved from the third cervical to the first thoracic vertebra (see Plate 2 and Plate 3). This alteration in position is thought to be due to the pull of m. latissimus dorsi upon the humerus, as its traction is not satisfactorily opposed by other muscles. The sternal end of the clavicle maintains its original position, with the result that the clavicle partially rotates, as seen in the X-ray, and in addition it develops an arch. Muscular changes were found in the animals that were deprived of their scapulae, as would be expected. In the region where the scapula had been there was found a dense plaque of fibrous tissue into which the muscles of extrascapular origin inserted. This tissue was roughly the size of the scapula, and very heavy. Its important feature was that it looked as though the muscles could perform most of their actions, through the mediation of the scar, and therefore retain at least a part of their functional integrity.

M. serratus anterior must be considered separately in discussion of the action of the shoulder girdle. This is especially true in this instance, for it concerns the ability of the muscle to perform useful movement through the mediation of the soft fibrous scar described above. In the first place, it must be remembered that the most important

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PLATE 1

Photographs of rats from which both scapulae have been removed. No. 1. Side view of animal showing abnormal prominence of elbows. Nos. 2, 3, 4. Views demonstrating abnormal prominence of the elbows, and tendency to keep fore feet together or crossed.

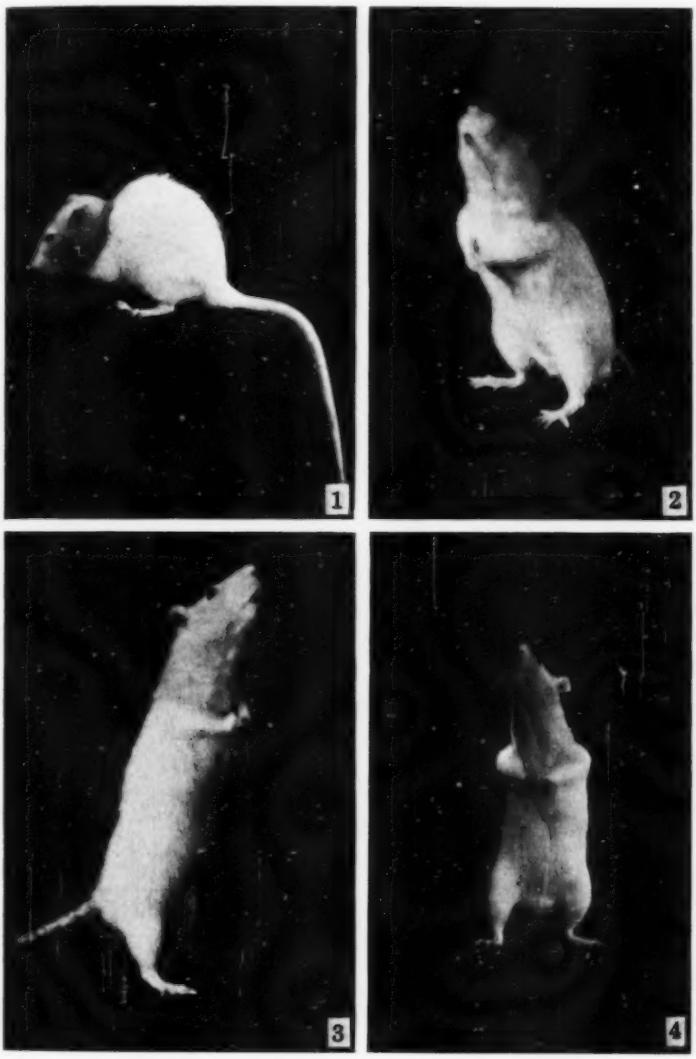
1
2
3
4

PLATE 2

Retouched X-rays of normal rat. No. 1. Antero-posterior view. Note level of shoulders at third cervical vertebra. Also, note arch of clavicles is concave anteriorly. No. 2. Lateral view of same animal.

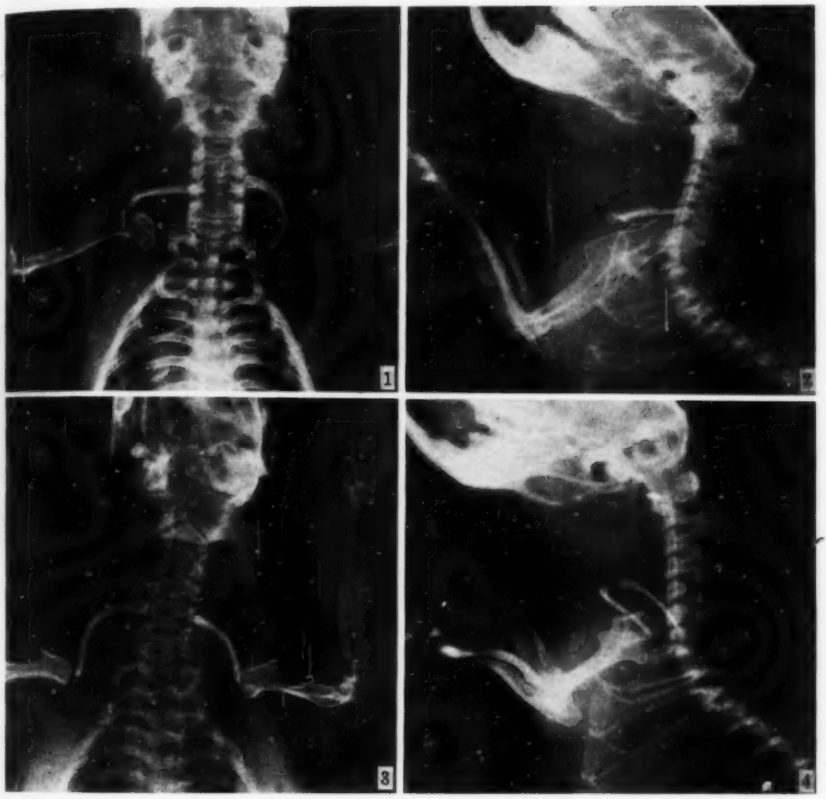

PLATE 3

Retouched X-rays of scapulectomized rats. Nos. 1 and 3. Antero-posterior views showing posterior displacement of shoulder, to level of the first thoracic vertebra and convex arch of the clavicles anteriorly. Note that in No. 1 there is no obvious deformity of the humerus, while in No. 2, there is marked reduction in the length of the humerus. Nos. 3 and 4. Lateral views of the corresponding rats, showing clavicular displacement and deformity.

functions of m. serratus anterior in the rat are to rotate and therefore to project the shoulder, and to form a sling in which the thorax rests (see fig. 1 and fig. 2). These functions are necessarily impaired by the loss of the scapula. Absence of the sling in which the thorax rests has a definite effect in forcing the animal to adopt bipedal gait at any time when quick motion must be resorted to, and in avoiding activities

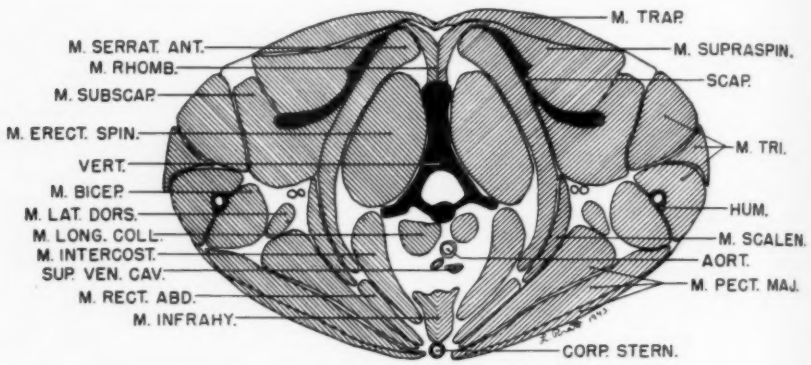

FIG. 1. DIAGRAMATIC VIEW OF SECTION THROUGH LOWER CERVICAL SPINE OF RAT, SHOWING THE MUSCULAR AND BONY RELATIONSHIPS IN THIS PART OF THE PECTORAL GIRDLE

Abbreviations: m. serrat. ant.—muscle serratus anterior; m. rhomb.,—muscle rhomboideus; m. subscap.—muscle subscapularis; m. erect. spin.—muscles erectores spinae; vert.—vertebra; m. bicep.—muscle biceps; m. lat. dors.—muscle latissimus dorsi; m. long. coll.—muscle longus colli; m. intercost.—muscle intercostalis; sup. ven. cav.—superior vena cava; m. rect. abd.—muscle rectus abdominus; m. infrahy.—infrahyoid musculature; m. trap.—muscle trapezius; m. supraspin.—muscle supraspinatus; scap.—scapula; m. tri.—muscle triceps; hum.—humerus; m. scalen.—muscle scaleneus; aort.—aorta; m. pect. maj.—muscles pectorales majores; corp. stern.—corpus sterni.

which bring undue strain upon the limbs. Although part of m. serratus anterior is left, it is not functionally useful because the scar is soft and flexible, and it is not possible to force it to push the arm foreward in projection. For this reason, it is useless in forming a hammock for the thorax, and is also unable to immobilize the shoulder properly. Only the posterior part of this muscle has any useful function, and that is the application of traction to the caudal part of the scar.

In general, the extrascapular muscles which have been considered are not able to function with their previous efficiency. Following section, they become shortened and fuse with the scar. This means that they are unable to contract to the same extent as previously because they are not fully stretched at the beginning of contraction. This materially reduces their efficiency, and when this is added to the impairment of function resulting from the elasticity of the scar, it is found that these alterations result in a grossly inefficient anatomical structure.

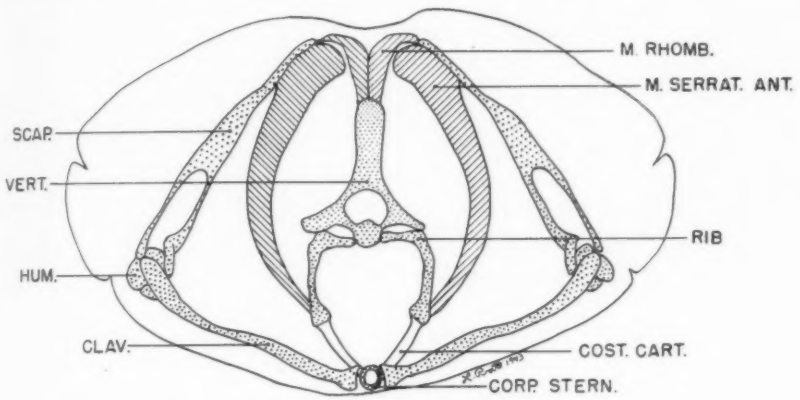

FIG. 2. SCHEMATIC REPRESENTATION OF THE SHOULDER ARCHITECTURE, SHOWING THE MUSCULAR HAMMOCK FORMED BY MUSCLE SERRATUS ANTERIOR, AND ITS BONY SUPPORTS, THE SCAPULA AND CLAVICLE, AND THEIR RELATIONS TO EACH OTHER

Muscle—cross hatch; bone—stipple; cartilage—clear. Abbreviations, as in fig. 1. Additional: cost. cart.—costal cartilage; rib—rib.

Individual muscles are described below, from dissection of fresh specimens.

In animal number 1, the posterior part of m. trapezius originated from the seventh cervical vertebral spine as a heavy tendon. However, all the muscular tissue arose from the sixth and above, and passed to the head of the humerus. M. sternocleidomastoideus was normal; it inserted upon the sternal end of the clavicle and also upon the manubrium sterni. The tendon of the latissimus dorsi was much narrower than in the control, and the muscle was much thinner and materially

more transparent. The rhomboidei were not identified. M. pectoralis major apparently was unaffected by the operation. M. pectoralis minor was quite normal in appearance, but it was inserted upon the head of the humerus. M. triceps was pale and whitish, as though invaded by a considerable amount of fibrous tissue. There was still left a considerable quantity of muscular fiber. M. serratus anterior was present, but small.

In the scapulectomized animals particular attention was paid to an examination of the muscles of the antebrachium and brachium, and a search was made for atrophy of the intrinsic musculature. In no instance were such changes found; hence it was concluded that no significant injury had been inflicted on the brachial plexus during the trauma of the operation.

Skeletal changes were noted in the scapulectomized animals. The clavicle developed an arch that is much more prominent than in normal animals. The humeri did not grow as large as in controls but this may have been partly due to interference with the circulation to the epiphysis of the head of the humerus at the time of operation. One animal developed humeri of markedly different lengths, indicating that at operation some traumatic damage was done that prevented normal development of the bone. Modification of the bones in the hind limbs was in the direction of the bipedal animals; in other ways, growth was normal.

CLEIDECTOMY

Method

Week old litters of rats were used, under ether anesthesia, as before; but, upon the infant rat, operation to remove the clavicle is a much more difficult and uncertain surgical procedure. The superficial external jugular passes directly over the clavicle, and joins the larger internal jugular immediately under this bone. In removing clavicles from week old rats, the lumen of this latter vessel is on occasion entered, usually with fatal result to the individual. The wall of the vessel at this stage is very thin, and as a result it is difficult to remove the bone without damaging it. The most successful method was to incise the chest above and over both clavicles. This incision allows for considerable retraction of the skin, and the clavicle may be easily seen. If it is divided at the junction of the middle and outer thirds, the proximal fragment may be removed with relative ease. This whole procedure, however, is uncertain and it cannot be done with the uniformly good results obtained in removing forelimbs or scapulae. If difficulties are avoided at operation, uneventful recovery is the rule. No postoperative infections occurred.

PLATE 4

Retouched X-rays of cleidectomized rat. No. 1. Antero-posterior view showing normal position of shoulders, at the level of the fourth cervical vertebra. The interhumeral distance is slightly reduced. No. 2. Lateral view of the same animal showing relatively normal shoulder architecture.

Results

The cleidectomized rat differs but little from the normal animal. In activity, there is almost no observable difference between the control and the rat from which the clavicles have been removed. In performing some special movements there is markedly increased flexibility in the shoulder of these animals, but there is no deficiency in their abilities. They climb rapidly, jump up or down, and have no difficulty breaking their fall when they jump from a shelf to the floor of the cage. They are not timid, as are those without scapulae, and in no way does the loss of the clavicle seem to weaken the functional architecture of the pectoral girdle.

Cleidectomy produces little change in the X-ray finding (see plate 4). The clavicles are not seen and the level of the head of the humerus is the same as in the normal rat. The humeri are slightly closer together than in controls, due to the normal tone of m. pectoralis major, which is unopposed by the clavicle; but there is no significant anatomical alteration.

The cleidectomized rat showed no morphological changes at autopsy, except for a well healed scar where the incision for removal of the clavicle was made. M. sternocleidomastoiedus was found to be firmly attached to the manubrium sterni and by scar to m. pectoralis major, which had become attached to the ribs and intercostal muscles, leaving no visible defect in muscle mass.

Bone growth and skeletal configuration was apparently unaffected by cleidectomy, little or no change being found in the skeletal system.

DISCUSSION

Experimental removal of the scapula or clavicle from newborn rats leads to certain modifications in their activity and morphology. In the case of excision of the scapula, modification is in the direction of the bipedal rat. The gait becomes hopping, and the animal uses its hind legs for all the work of life. Interestingly, however, the animal which still has its front limbs attached does not develop the assured and confident method of travel that the biped soon acquires. The presence of inefficient forelimbs leads to reliance being placed upon them, which in turn precludes the development of an efficient hopping locomotion by the hind limbs. The bipedal rat has no air of timidity in any of its

activities, but the scapulectomized animal is exceedingly cautious at all times.

In posture, the scapulectomized rat is singular, as the void left by absence of the scapula gives undue prominence to the elbows, and the deformity characteristic of this animal is crossed fore feet, coupled with protruding elbows. The internal rotation of the fore limb, resulting in crossing of the fore feet, is due chiefly to the unopposed tone of *m. pectoralis major*. The function of the scapula in the pectoral appendage is most important, as it gives stability to all motions, especially those involving extension of the limb.

In its absence, lacking the ability to push with the fore limbs, the animal does not do much of any walking with the front feet. The fore feet are used to grasp the bars of the cage, while the work of climbing is done by the hind legs, and in some instances the chin is used in place of the fore feet as a means of stabilization. Speed is much reduced; travel is difficult and slow. From this it is seen that the scapula is an important member of the pectoral girdle, lending most of the stability and solidity to functions performed by the fore limbs.

A prerequisite for proper action of the pectoral appendage is adequate support of the fore limb. This is accomplished chiefly by *m. serratus anterior*. In speedy cursorial animals, like the ungulates, the clavicles are either much reduced in size, or even are lacking. In these animals, this support is largely the result of *m. serratus anterior*. The heavy muscular hammock supports the weight of the animal and takes up the strains of running, and of landing following a jump. The support for the muscular hammock is the scapula which is held in proper position by the *trapezius* and the *rhomboid* muscles at the vertebral margin, and the clavicle at the humeral end. In animals that lack clavicles, maintainance of the interhumeral distance is achieved by the pectorals.

In order to analyze the actions of these muscles and bones, it has been necessary to oversimplify. It must be remembered that other muscles have actions relative to the functions described above, but their contributions are less important than those of the described muscles.

The clavicle is not a very important bone in the pectoral appendage of the rat, as its excision at an early age does not interfere with locomotion, growth or activity of the animal, and alters its morphological characters remarkably little. In general, reduction in the size of the

clavicle in the mammalia is found in species in which the fore limbs are used only for locomotion. In some animals, like the ungulates, the small clavicles are associated with the ability to attain high speeds. It is difficult to say whether or not this effect is produced in the rat, but it can be stated that absence of the clavicle does not handicap the animal. In the case of removal of the scapula, a definite, gross reduction in speed is noted, but this is not seen in connection with absence of the clavicle. Locomotion seems approximately the same in these rats as in control litters. Great flexibility of the pectoral appendage is noted, especially when the animal is performing acts of grooming or in other acts in which it passes the fore feet behind the ears, and it is easy to push the scapula and humerus far out of their normal positions. At such times as this, the flexibility of motion is especially noticeable. In other ways, these rats show no significant alteration.

CONCLUSIONS

Bilateral scapulectomy and cleidectomy were performed upon newborn rats. From study of these animals it was found that the scapula is essential in the maintenance of a functionally useful pectoral appendage in the rat, while removal of the clavicle did not reveal such importance connected with this bone. The animal lacking the scapula was timid, and not nearly as active as the control, or as the bipedal animal. Deformity consisting of a crossing of the forelimbs and abnormal prominence of the elbows was observed. The pectoral appendage is displaced from the level of C-3 to T-1 with rotation and bending of the clavicle. No significant alteration in the cleidectomized animal was noted, either in function or in morphology, and it was decided that the clavicle is not an important unit in the functional or morphological development of the pectoral appendage.

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